Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study

Theodora Psaltopoulou, Androniki Naska, Philippos Orfanos, Dimitrios Trichopoulos, Theodoros Mountokalakis, and Antonia Trichopoulou

ABSTRACT
Background: Diet has been reported to influence arterial blood pressure, and evidence indicates that the Mediterranean diet reduces cardiovascular mortality.
Objective: The objective was to examine whether the Mediterranean diet, as an entity, and olive oil, in particular, reduce arterial blood pressure.
Design: Arterial blood pressure and several sociodemographic, anthropometric, dietary, physical activity, and clinical variables were recorded at enrollment among participants in the Greek arm of the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Of these participants, 20343 had never received a diagnosis of hypertension and were included in an analysis in which systolic and diastolic blood pressure were regressed on the indicated possible predictors, including a 10-point score that reflects adherence to the Mediterranean diet and, alternatively, the score’s individual components and olive oil.
Results: The Mediterranean diet score was significantly inversely associated with both systolic and diastolic blood pressure. Intakes of olive oil, vegetables, and fruit were significantly inversely associated with both systolic and diastolic blood pressure, whereas cereals, meat and meat products, and ethanol intake were positively associated with arterial blood pressure. Mutual adjustment between olive oil and vegetables, which are frequently consumed together, indicated that olive oil has the dominant beneficial effect on arterial blood pressure in this population.
Conclusions: Adherence to the Mediterranean diet is inversely associated with arterial blood pressure, even though a beneficial component of the Mediterranean diet score—cereal intake—is positively associated with arterial blood pressure. Olive oil intake, per se, is inversely associated with both systolic and diastolic blood pressure. Am J Clin Nutr 2004;80:1012–18.

KEY WORDS Olive oil, Mediterranean diet, arterial blood pressure, European Prospective Investigation into Cancer and Nutrition study, EPIC study, Greece

INTRODUCTION
Hypertension can result in stroke, myocardial infarction, congestive heart failure, sudden cardiac death, peripheral vascular disease, and renal insufficiency, and yet it is clearly modifiable (1). Guidelines for the management of hypertension emphasize dietary factors (2, 3). Adoption of the low-saturated fat, plant-based DASH (Dietary Approaches to Stop Hypertension) diet has been advocated together with weight reduction, dietary sodium reduction, enhancement of physical activity, and moderation in alcohol consumption (2). An increase in fruit, vegetable, and fish intakes and a reduction in saturated fat and cholesterol intakes has also been advocated by the European Society of Hypertension (3).

The Mediterranean diet has been considered to be a healthy eating pattern ever since Ancel Keys initiated the Seven Countries Study in the 1950s (4, 5). Several studies have indicated that adherence to a Mediterranean diet is associated with a reduction in total and cardiovascular mortality (6-9). High intakes of olive oil are considered a hallmark of the traditional Mediterranean diet, resulting in high intakes of monounsaturated fatty acids and lower intakes of saturated fatty acids. Replacement of saturated with monounsaturated lipids is associated with a considerable reduction in coronary heart disease risk, through a mechanism involving reduction of LDL cholesterol, without a reduction of HDL cholesterol or an increase in triacylglycerols (10). Less is known about the relation of arterial blood pressure with Mediterranean diet or its dominant components. In a cross-sectional study of 2282 residents of the Attica area in Greece (which surrounds and includes the capital city of Athens), it was reported that adherence to a Mediterranean diet increases the likelihood of having the arterial blood pressure controlled (11).

To further evaluate the association between a Mediterranean diet and its components and systolic and diastolic blood pressure, we conducted a general population study in a large sample that covers most of the geographic regions of Greece. To avoid problems generated by diet modification subsequent to the diagnosis

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of hypertension, we excluded all persons who reported a diagnosis of hypertension at any time in the past. We ascertained diet through a validated, extensive food-frequency questionnaire and we controlled in the analysis for several factors with confounding potential, including total energy intake and physical activity.

SUBJECTS AND METHODS

Subjects
The study sample consisted of volunteers aged 20–86 years, who were recruited during a 5-y period (1994–1999) from around Greece to participate in the Greek component of the EPIC study (European Prospective Investigation into Cancer and Nutrition). EPIC is a multicountry, prospective study conducted in 22 research centers in 10 European countries and coordinated by the International Agency for Research on Cancer (IARC) to examine the role of dietary, biological, lifestyle, and environmental factors in the etiology of cancer and other chronic diseases. Details on the design and methods of the EPIC study and the Greek cohort were previously described in detail (9, 12, 13). All participants signed an informed consent form before enrollment. The study protocol was approved by the ethics committees of IARC and the University of Athens Medical School. A total of 28572 volunteers were enrolled in the Greek EPIC cohort, but 1750 of them (6.1%) were excluded because of missing values for one or more variables used in the present analysis. Of the remaining 26822 participants, 6479 (24.2%) of them had already received a diagnosis of hypertension and were also excluded because they may have changed their diet in response to that diagnosis. Thus, 20343 persons were included in the current study.

Methods

Standard interviewing procedures were used to assess sociodemographic characteristics, such as age, place of residence, and years of schooling. Anthropometric measurements also followed standard procedures and were taken with subjects wearing light clothing and no shoes. Body weight was measured to the nearest 100 g and height to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (in kg) over height squared (in m²). Waist and hip circumferences were measured with an inelastic tape and were recorded to the nearest 0.1 cm.

Usual dietary intake over the past year was assessed through a validated, semiquantitative, interviewer-administered food-frequency questionnaire (14, 15). The questionnaire included ≈150 food items and beverages as well as questions on habitual cooking methods and type of lipids used in cooking. Standard portion sizes were used for the estimation of consumed quantities (14, 15). A gradient of adherence to the traditional Greek-Mediterranean diet was constructed on the basis of 9 nutritional components (6, 9). Values of 0 to 1 were assigned to each of the 9 indicated components by using the respective sex-specific medians as cutoffs (9). Specifically, persons with a below the median consumption of components with a presumably beneficial effect on overall mortality (vegetables, legumes, fruit, cereals, and fish) were assigned a value of 0, whereas persons with consumption above the median were given a value of 1. In contrast, persons with a below the median consumption of components with a presumably detrimental effect on overall mortality (meat, meat products, and dairy products, which are rarely low-fat in Greece) were assigned a value of 1, whereas persons whose consumption of these components was above the corresponding median were given a value of 0. For ethanol, a value of 1 was given to men whose consumption of ethanol was from 10 to <50 g/d, whereas for women the corresponding cutoffs were 5 and 25 g/d (9). Finally, for lipid intake, the ratio of monounsaturated to saturated lipids instead of the ratio of polyunsaturated to saturated lipids was used, because monounsaturated lipids are consumed in much higher quantities in Greece. Thus, a 10-point Mediterranean diet scale was constructed, which could take a value from 0 (minimal adherence to the traditional Mediterranean diet) to 9 (maximal adherence to the traditional Mediterranean diet).

Professional and leisure time physical activity were assessed by a special section of the lifestyle EPIC questionnaire (13, 16). Briefly, the average time per day spent on household, professional, sporting, and other activities was calculated. A metabolic equivalent index was computed by assigning a multiple of resting metabolic rate (17) to each activity (MET value). Time spent on each of the activities was multiplied by the MET value of the activity, and all MET-hour products were summed to give a total daily MET score, which represented the amount of energy per kilogram body weight expended during an average day.

Years of schooling was used as a proxy to socioeconomic status. Type of residence was determined according to the population of the area the person was living in. Urban areas were classified as those having >10000 inhabitants and, rural areas (including semiurban) with ≤9999 inhabitants (18).

Arterial blood pressure measurements were conducted by specially trained physicians with the use of a mercury sphygmomanometer (Baumanometer; WABAum Co. Inc, New York). Participants were seated on a chair with their backs supported and their right arm bared at the level of the heart. After 5 min of rest, systolic and diastolic blood pressures were measured twice, with at least a 2-min interval between the 2 measurements. The averages of the 2 readings for both systolic and diastolic blood pressure were used. As indicated, persons who reported a diagnosis of hypertension at any time in the past or who were using anti-hypertensive drugs were excluded from the analysis.

Statistical analysis

Systolic and diastolic blood pressure were alternatively regressed on age (continuously, expressed per 10-year increment); sex; place of residence; interaction terms of the former 3 variables, 2 at a time; years of schooling (continuously, expressed per 3-year increment); BMI (continuously, per SD); waist-to-hip ratio (continuously, per SD); energy intake (continuously, per SD); physical activity (continuously, per SD); and Mediterranean diet score (continuously, expressed per 3 unit increment). In additional models, the components of the Mediterranean diet score, as well as olive oil, were alternatively substituted for the Mediterranean diet score (all of them continuously per SD increment, except for ethanol intake). In all instances, SDs were sex-specific. The STATA statistical package was used for the analysis (Intercooled Stata 7.0 for WINDOWS 98/95/NT; STATA Corporation, College Station, TX).

RESULTS

The distribution of 8685 men and 11 658 women without a prior diagnosis of hypertension, by systolic and diastolic blood
pressure in the Greek EPIC study, is shown in Table 1: 2666 (30.7%) men and 2758 (23.7%) women had a systolic or diastolic blood pressure that would classify them as probable hypertensives, notwithstanding the absence of a relevant diagnosis. In Table 2, representative values of the participants’ age, Mediterranean diet score, and variables considered to be predictive of hypertension (ie, years of schooling, BMI, waist-to-hip ratio, energy intake, and physical activity expenditure) are shown. The latter variables are potential confounders in the association between qualitative aspects of diet and arterial blood pressure.

The mean (±SD) daily consumption of olive oil and nutritional variables that contribute to the Mediterranean diet score, by sex, are given in Table 3. In the same table, the cutoffs used to calculate the Mediterranean diet score are indicated. The unusually high consumption of vegetables and the high consumption of olive oil in the Greek population are evident in these data. Indeed, these 2 variables are highly correlated, the Spearman correlation coefficient being 0.7. This value is a numerical expression of the tendency in the Greek population to use olive oil for the preparation of vegetable dishes (raw or cooked).

Mutually adjusted partial regression coefficients of systolic and diastolic blood pressure, respectively, on the indicated predictor variables are shown in Tables 4 and 5. As expected, arterial blood pressure declines significantly with increasing educational level and increases significantly with increasing BMI and waist-to-hip ratio. The Mediterranean diet score is significantly inversely associated with both systolic and diastolic blood pressure, after control for sociodemographic and anthropometric variables as well as for energy intake and energy expenditure. Substituting the various components of the Mediterranean diet score and olive oil for the score in the regression showed that olive oil, vegetables, and fruit are the principal factors that explain the overall effect of the Mediterranean diet on arterial blood pressure. It is worth noting that cereals, a nutritional factor generally considered to be beneficial to health, are positively associated with both systolic and diastolic blood pressure, after control for sociodemographic and anthropometric factors.

TABLE 2
Sociodemographic and anthropometric characteristics, energy intake, and physical activity level in men and women without a prior diagnosis of hypertension in the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study, 1994–1999

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 8685)</th>
<th>Women (n = 11 658)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediterranean diet score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years of schooling (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kcal)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity (MET h/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 All values are x ± SD, MET, metabolic equivalents.

TABLE 3
Daily food consumption in men and women without a prior diagnosis of hypertension in the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study, 1994–1999

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 8685)</th>
<th>Women (n = 11 658)</th>
<th>Cutoff¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables (g)</td>
<td>582.3 ± 233.0</td>
<td>549.9</td>
<td>542.3 ± 236.7</td>
</tr>
<tr>
<td>Legumes (g)</td>
<td>10.5 ± 7.5</td>
<td>9.1</td>
<td>8.0 ± 5.9</td>
</tr>
<tr>
<td>Fruit (g)</td>
<td>372.4 ± 212.6</td>
<td>362.5</td>
<td>377.7 ± 212.2</td>
</tr>
<tr>
<td>Dairy products (g)</td>
<td>224.9 ± 150.8</td>
<td>196.7</td>
<td>222.8 ± 150.9</td>
</tr>
<tr>
<td>Cereals (g)</td>
<td>190.6 ± 82.4</td>
<td>177.7</td>
<td>144.0 ± 55.8</td>
</tr>
<tr>
<td>Meat and meat products (g)</td>
<td>129.6 ± 61.7</td>
<td>120.8</td>
<td>95.8 ± 45.9</td>
</tr>
<tr>
<td>Fish and seafood (g)</td>
<td>26.5 ± 20.1</td>
<td>23.7</td>
<td>22.1 ± 15.1</td>
</tr>
<tr>
<td>Olive oil (g)</td>
<td>83.7 ± 24.0</td>
<td>—</td>
<td>76.6 ± 21.8</td>
</tr>
<tr>
<td>Monounsaturated: saturated lipids</td>
<td>1.8 ± 0.5</td>
<td>1.7</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Ethanol (g)²</td>
<td>19.0 ± 24.7</td>
<td>10–50</td>
<td>3.7 ± 6.4</td>
</tr>
</tbody>
</table>

¹ The beneficial effect is assumed in the indicated range.
² Values of the sex-specific medians in the Greek EPIC cohort (9).
associated with both systolic and diastolic blood pressure, as is a high intake of ethanol. The consumption of meat and meat products is significantly positively associated with diastolic blood pressure, whereas the consumption of fish and seafood is significantly inversely associated with systolic blood pressure.

Because the intakes of vegetables and olive oil are highly correlated and because they are both inversely associated with arterial blood pressure, we examined whether their apparent effects in Tables 4 and 5 are mutually confounded. Introduction of both olive oil and vegetables in the models indicated in Tables 4 and 5 showed that the effect of olive oil was not substantially affected by the control for vegetable intake (the partial regression coefficient for systolic blood pressure decreased in absolute terms from −0.84 to −0.83 and for diastolic blood pressure from −0.36 to −0.25). In contrast, the effect of vegetables was considerably less, in absolute terms, when olive oil was controlled for (the partial regression coefficient for systolic blood pressure changed from −0.45 to −0.01 and for diastolic pressure from −0.35 to −0.22).

We repeated our analyses by substituting waist circumference for waist-to-hip ratio, by adding tobacco smoking among the core variables, and by controlling for dieting for any reason at the time the subjects were examined. When waist circumference was controlled for, instead of waist-to-hip ratio, the only regression coefficients that were substantially affected were, as expected, those for BMI, whereas no noticeable changes were evident with respect to the regression coefficients for Mediterranean diet score, its components, or olive oil. Both tobacco smoking and dieting for any reason were significantly inversely associated with both systolic and diastolic blood pressure, but, again, none of these variables were found to confound the association between Mediterranean diet score, its components, or olive oil on the one hand and systolic or diastolic blood pressure on the other hand.

We examined the association between arterial blood pressure and a variant of the Mediterranean diet score, in which a high intake of cereals was considered to be detrimental (value = 0) and a low intake of cereals as beneficial (value = 1). As expected, the regression coefficient of systolic blood pressure per 3 units of Mediterranean diet score changed from −0.8 (Table 4) to −1.0, and the regression coefficient of diastolic blood pressure for the same increment changed from −0.2 (Table 5) to −0.4 (P for both < 0.001).
DISCUSSION

In a large, general, population-based study, we found evidence that adherence to a Mediterranean diet, as operationalized through the Mediterranean diet score, is inversely associated with both systolic and diastolic blood pressure. The values of the regression coefficients, which describe the average change in the systolic and diastolic blood pressure per specified increment in the Mediterranean diet score, are small. Because, however, arterial blood pressure is characteristic of all persons, small individual changes are translated into substantial changes in morbidity from hypertension-related diseases, including coronary heart disease and stroke, at the population level (19, 20). Of the components of the Mediterranean diet, vegetables, fruit, and olive oil (as reflected in the high ratio of monounsaturated to saturated lipids), are mainly responsible for the apparent protection against hypertension conveyed by the Mediterranean diet. The high content of plant foods in minerals, which tend to reduce arterial blood pressure (including potassium, magnesium, and calcium), may represent mediating mechanisms of the apparent protective effects of these foods, whereas the high content of plant foods and olive oil in antioxidants may also contribute to the health of the vascular system (21-24). Compatible with the apparent effect of the Mediterranean diet score is the detrimental effect associated with the intake of meat and meat products (significant with respect to diastolic blood pressure), the beneficial effect associated with the intake of fish and seafood (significant with respect to systolic blood pressure), and the detrimental effect associated with a high intake of ethanol. A high intake of alcoholic beverages has been consistently associated with high arterial blood pressure (25, 26), whereas meat intake has been reported to increase and fish intake to decrease arterial blood pressure (27, 28). Note, however, that one of the beneficial components of the Mediterranean diet, cereal consumption, is actually positively associated with both systolic and diastolic blood pressure, possibly because carbohydrate intake has been linked to several cardiovascular disease risk factors and clinical entities (29, 30) and because salt is frequently added to cereal products, particularly bread, which is a widely consumed staple food in Greece. Indeed, it may be worth examining in future studies the data-derived evidence that cereal intake is positively associated with arterial blood pressure.

The Mediterranean diet shares many of the characteristics of the DASH diet, which is widely recommended in the United States. The main difference between the 2 diets is that the Mediterranean diet is high in olive oil (2, 31, 32). Simply put, it could be argued that the DASH diet, which is enriched in olive oil rather than in other fatty acids and perhaps in some grain products, could represent an improvement over the classic DASH diet, provided that the balance between total energy intake and expenditure is preserved and the dietary pattern is culinary acceptable. On the basis of the Greek EPIC data, it is difficult to argue whether olive oil or monounsaturated lipids in general have differential effects on arterial blood pressure.

There is considerable evidence that the consumption of fruit and vegetables is inversely associated and the consumption of cereals and meat and meat products is positively associated with arterial blood pressure (20, 33, 34). Evidence suggests, but does not conclude, that fish intake is inversely associated with arterial blood pressure (27, 35). The results of the few comprehensive studies that have been conducted to evaluate the association between arterial blood pressure and intake of dairy products suggest an inverse association (36). In contrast, there is wide agreement that a high consumption of alcoholic beverages is positively associated with arterial blood pressure (25, 26).

A Mediterranean diet is widely regarded as a health-promoting diet, in terms of both general and cardiovascular mortality (4, 6, 9, 37, 38). The search for mediating processes has mainly focused on the blood lipid profile and mechanisms of thrombogenesis (29, 39). Few studies have examined the relation of olive oil or a Mediterranean diet with arterial blood pressure. The fatty acids in olive oil are protected by natural antioxidants (40), including carotenoids, tocopherols, and phenolic compounds. Antioxidants tend to inactivate the effects of free radicals and lipid peroxidation, which could affect arterial stiffness (41-44). In animal experiments, olive oil has been compared with sunflower oil, which is more susceptible to oxidation (45). It has been shown that olive oil decreased arterial blood pressure more than did sunflower oil, an effect that was attributed, at least in part, to olive oil’s polyphenolic content (21). In a recent publication, Alemany et al reported that intraperitoneal or oral administration to animals of 2-hydroxyoleic acid, a synthetic derivative of oleic acid, which is the primary monounsaturated fatty acid found in olive oil, induced substantial decreases in arterial blood pressure, mainly systolic blood pressure (46).

In humans, olive oil was again compared with sunflower oil and was found to reduce the need for daily pharmaceutical antihypertensive treatment. According to Ferrara et al (47), daily doses of blood pressure medication were reduced by 48% during the olive oil diet and by 4% during the sunflower oil diet; this finding could be attributed to polyphenols, which enhance nitric oxide concentrations and may help dilate arteries, which reduces blood pressure. Polyphenols are completely absent in sunflower oil. In an epidemiologic study undertaken in Greece, a Mediterranean diet—operationalized in line with the recommendations of Trichopoulou et al (6, 48, 49)—was found to be inversely associated with arterial blood pressure (11). The current study, however, is considerably larger, and its design allowed both the control for a large set of potential confounders and a separate examination of the specific effects of the various components of the Mediterranean diet.

The strengths of this study were its large sample size, reliance on a validated food-frequency questionnaire, coverage of a large set of potential confounders, and exclusion of persons with a diagnosis of hypertension, the latter of which prevents bias that could be introduced from changes in habitual diet in response to the diagnosis of hypertension. The cross-sectional nature of the study was a drawback; however, the results of our evaluation of the association of arterial blood pressure with socioeconomic status, BMI, and waist-to-hip ratio indicate that overt biases were not operating in the study.

Salt intake is one of the principal dietary components involved in increases in arterial blood pressure, but it is difficult to ascertain (30). Thus, a conceivable relation between salt intake and Mediterranean diet could not be evaluated in our study. Dietary recommendations to manage hypertension, such as the DASH diet, are, however, considered independently of advice to reduce salt intake (2).

In conclusion, we found evidence that a Mediterranean diet, which shares many of the characteristics of the DASH diet, is inversely associated with both systolic and diastolic blood pressure. Olive oil intake per se may be as important as fruit and...
vegetable intakes in the apparent beneficial effect of the Mediterranean diet in the context of arterial blood pressure control.

TP took the lead in the development of several aspects of the manuscript. PO coordinated the data analysis. AN contributed to the data collection and processing. TM was the clinical consultant for hypertension. DT was the epidemiology consultant. AT was the principal investigator of the Greek EPIC project and supervised all aspects of this project. All authors contributed to the writing of the manuscript. None of the authors declared a conflict of interest.

REFERENCES


45. Quiles JL, Huertas JR, Battino M, et al. The intake of fried virgin olive


Quercetin, fruit consumption, and bone mineral density

Dear Sir:

McGartland et al (1) reported a correlation between a diet high in fruit and bone mineral density. Their article includes a discussion of how a low pH stimulates osteoclasts and how fruit’s alkaline-forming properties influence the body’s acid-base balance.

Another mechanism exists that could work in parallel or in synergy with the one proposed by McGartland et al. Diets high in fruit contain high amounts of flavonoids (2). The flavonoid quercetin decreases the differentiation of osteoclast progenitor cells and inhibits the activity of mature osteoclasts (3–5). Quercetin might act together with the alkaline-forming properties of fruit to inhibit osteoclasts and enhance bone mineral density.

The author had no conflicts of interest to report.

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REFERENCES

Reply to CM Ross

Dear Sir:

In her response to our article “Fruit and vegetable consumption and bone mineral density: the Northern Ireland Young Hearts Project,” Ross raises an interesting issue for discussion. We agree that the flavonoid quercetin may inhibit osteoclasts and enhance bone mineral density.

Phytoestrogens comprise a variety of structurally diverse chemicals, with flavonoids as their largest group (1). In our discussion, we stated that phytoestrogens have been identified as being potentially important for bone health (2). Because we did not measure quercetin concentrations in our subjects, we did not specifically mention quercetin in our discussion. We thank Ross for her interest in our article and for highlighting the potential role of quercetin in bone health.

The authors had no conflicts of interest to report.

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REFERENCES
Dear Sir:

We read with great interest the excellent article by Specker (1), who carried out a comprehensive review of studies that investigated material and neonatal outcomes of vitamin D deficiency or supplementation during pregnancy. We would like to add to this discussion an important topic that has become evident recently and that was not addressed by the clinical studies that Specker reviewed: the fact that extrarenal synthesis of 1,25-dihydroxyvitamin D [1,25(OH)₂D₃, or calcitriol] is of great importance for homeostasis in a multitude of tissues, including the immune system, and that this function of vitamin D is likely responsible for the numerous epidemiologic observations that persons who live at higher latitudes, who are more prone to vitamin D deficiency, are at increased risk of not only developing prostate, colon, breast, and other solid tumors; hypertension; and cardiovascular heart disease but also of developing autoimmune diseases, including multiple sclerosis and type 1 diabetes (2–5). Consequently, increasing evidence now indicates that vitamin D deficiency during pregnancy may represent for the fetus a predisposing factor for the future development of a broad variety of diseases, including those of the immune system, such as atopic dermatitis or autoimmune diseases (4, 6, 7).

During the past few years, important new immunomodulatory effects of vitamin D analogues have been characterized (4, 7–9). It has been shown that various cell types involved in immunologic reactions (eg, monocytes, T and B lymphocytes, and Langerhans cells) not only express the vitamin D receptor but also possess the enzymatic machinery (25-hydroxyvitamin D-1α-hydroxylase) for the local synthesis of 1,25-dihydroxyvitamin D [1,25(OH)₂D₃, or calcitriol] of great importance for homeostasis in a multitude of tissues, including the immune system, and that this function of vitamin D is likely responsible for the numerous epidemiologic observations that persons who live at higher latitudes, who are more prone to vitamin D deficiency, are at increased risk of not only developing prostate, colon, breast, and other solid tumors; hypertension; and cardiovascular heart disease but also of developing autoimmune diseases, including multiple sclerosis and type 1 diabetes (2–5). Consequently, increasing evidence now indicates that vitamin D deficiency during pregnancy may represent for the fetus a predisposing factor for the future development of a broad variety of diseases, including those of the immune system, such as atopic dermatitis or autoimmune diseases (4, 6, 7).

In conclusion, a growing body of evidence now clearly indicates that vitamin D deficiency during pregnancy may be a predisposing factor not only for fetal growth and bone metabolism but also for correct development of the fetal immune system.

The authors had no conflicts of interest to report.

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REFERENCES


Reply to J Reichrath and K Querings

Dear Sir:

I appreciate the interest of Reichrath and Querings in my review of vitamin D requirements during pregnancy. Those authors noted that there is increasing evidence that vitamin D deficiency during pregnancy may lead to a predisposition to immunologic diseases, yet none of the studies they cited related pregnancy. However, one study was found in the literature that investigated the relation between vitamin D status during pregnancy and the subsequent development of immunologic diseases (1). This case-control study (85 cases and 1071 controls) from Norway found that cord liver oil taken during pregnancy was associated with reduced risk of type 1 diabetes in the offspring. These investigators, however, did not find an association between type 1 diabetes among the offspring and the prenatal use of multivitamin supplements, which typically contain significant amounts of vitamin D. This association, therefore, is not likely to be a direct result of maternal vitamin D status, but could be due to additional nutritive factors in cord liver oil or other confounding factors. Although the studies discussed by Reichrath and Querings are intriguing, it would be premature to state that there is evidence that vitamin D deficiency during pregnancy may be a predisposing factor for the fetus to the development of immunologic diseases.

The author had no conflicts of interest to report.

Jörg Reichrath
Kerstin Querings

Bonny Specker
Absorption of iron from ferritin

Dear Sir:

I would like to comment on the article “Iron in ferritin or in salts (ferrous sulfate) is equally bioavailable in nonanemic women,” by Davila-Hicks et al (1). The conclusion indicated in the title is based on measurements of iron absorption from horse spleen ferritin that was radiolabeled in vitro and appears to contrast with the results of others whose studies using ferritin radiolabeled in vivo were not cited (2–4). For example, Skikne et al (4) also found that iron from ferritin radiolabeled in vitro was absorbed similarly to iron from ferrous sulfate. However, the same group further reported that radiiodirn incorporated into bovine spleen ferritin in vivo was significantly less absorbed than was iron from ferrous sulfate: 3.2% compared with 8.2% from a 3-mg dose with food, 3.8% compared with 24.1% from a 3-mg dose without food, 0.6% compared with 2.6% from a 50-mg dose with food, and 0.7% compared with 7.9% from a 50-mg dose without food (4). Those who have studied ferritin radiolabeled in vivo have concluded that ferritin iron is poorly absorbed and that it is not part of the nonheme pool of dietary iron that is readily exchangeable in and is similarly absorbed from the intestinal lumen (2–4). For instance, in vivo–labeled ferritin 59Fe was only 36% as well absorbed as was 59Fe from intrinsically labeled soybeans consumed in the same meal (2). It is possible that a lower absorption of ferritin iron may explain the slightly greater (10%) absorption of nonheme iron from extrinsically than from intrinsically labeled foods (5), which suggests that the ferritin iron content of food is only a minor portion of total food iron. It is worth noting that the ferritin iron content of foods has not been widely determined because of the lack of species-specific antibodies as well as the insolubility and possible time-dependent molecular changes that may make ferritin iron less exchangeable (6).

Each labeling method has potential problems. On the one hand, the in vivo labeling of animal ferritin has in some (2, 4), but not in all (3), reports involved procedures to limit the radiolabel incorporation into blood by reducing erythrocyte synthesis or increasing erythrocyte breakdown, and it is not known whether these techniques alter ferritin isomerization. It is clear that in the in vivo procedure does not uniformly label all of the iron in ferritin, but this would not necessarily explain the reduced iron bioavailability because the portion that is unlabeled may be less, not more, exchangeable or absorbable. On the other hand, in vitro labeling results in higher bioavailability that is unlabeled may be less, not more, exchangeable or absorbable.

In conclusion, research on iron bioavailability from ferritin labeled in vitro must be interpreted with caution. The evidence does not support the conclusion that iron absorbed from ferrous sulfate labeled a full range of isoferritins but that isotope incorporation into the more acidic forms was slightly higher (4). It is unlikely that horse spleen ferritin labeled with extra phosphorus in vitro (1) is comparable to plant ferritin. Using Mössbauer spectroscopy, Ambe et al (7) found that the form of ferric iron, representing ≈95% of the iron in soybeans, was clearly distinguishable from, but more similar to, horse spleen ferritin than to ferric phytate. Although physicochemical methods detected only minor alterations in ferritin labeled in vitro (1, 4), the human absorption results provide a distinguishing bioassay for ferritin labeled in vitro compared with in vivo.

Davila-Hicks et al (1) concluded that iron from ferritin or ferrous sulfate follow different metabolic pathways after absorption. This was based on similarities in iron absorption when measured by whole-body scintillation counting (22% and 22% from ferritin and ferrous sulfate, respectively; see Table 1) but differences in absorption when measured from erythrocyte iron incorporation (27% and 48%). The greater retention of isotope in the erythrocytes than in the whole body suggests methodologic difficulties. The specific method and assumptions used were not delineated. With the use of commonly used methods (see citations in reference 9) and an assumption of 80% incorporation of the absorbed isotope into blood, we repeatedly obtained similar absorption results between the 2 methods, including results with added ferrous sulfate (9). For instance, nonheme-iron absorption from a hamburger meal supplemented with 20 mg Fe as ferrous sulfate was 8.4% (geometric mean ± 1 SE: 6.8, 10.3) by whole-body counting (see data in Figure 1 of reference 9) and 8.5% (6.7, 10.7) by the erythrocyte incorporation method, and the assumption of 80% incorporation of the absorbed isotope into blood was confirmed (9). Note that the blood incorporation data in reference 9 was incorrectly labeled as the incorporation of the ingested rather than of the absorbed isotope dose; an erratum was submitted. Davila-Hicks et al (1) appear to have expressed the blood incorporation as a percentage of the ingested dose; expressed as such, their values seem excessive, but when expressed as the percentage absorbed seem insufficient.

With the use of data from individual subjects (n = 23), the blood incorporation method was highly correlated with the whole-body counting method (R² = 0.98) (9). These data do not confirm the finding that iron from ferrous sulfate is more extensively incorporated into blood than is apparent from whole-body counting measurements.

In conclusion, research on iron bioavailability from ferritin labeled in vitro must be interpreted with caution. The evidence does not support the conclusion that iron absorbed from ferrous sulfate...
follows a metabolic distribution different from that of iron absorbed from ferritin.

There were no conflicts of interest.

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Reply to JR Hunt

Dear Sir:

The comments of Hunt about our recently published article on the absorption of iron from ferritin (1) reflect the confusion that often exists over the consequences of the unique features of ferritin biology and chemistry: a solid mineral inside an organic protein shell. With the exception of calcium phosphate minerals, there is no other solid mineral in humans. The phase transition of calcium, phosphate, iron, and hydroxide from liquid to solid in biology remains poorly understood at this time.

Under physiologic conditions, the iron in solutions of ferritin—the protein with the solid iron mineral inside—appears to be physically in the same phase as iron in solutions of other nutritional, nonheme-iron complexes such as ferrous sulfate and ferric EDTA. However, the reality is much more complicated. Adding iron to ferritin involves a phase transition that makes full equilibration of exogenous iron very slow (2). In addition, ferritin protein varies in different tissues or under different physiologic conditions and controls the entry and exit of iron to and from the solid phase (3). Finally, the structure of the iron mineral is sensitive to physiologic conditions (4).

The discussion of the causes of the apparent inconsistencies in the results of using isotopically labeled ferritin in iron-absorption experiments appears in our first article on iron absorption from ferritin (5) and again in a recent review (6). Briefly, when labeled iron atoms are added to the unlabeled, solid mineral in ferritin, equilibration is very slow (days to months). In the 1997 study annotated in Hunt’s letter (7), the label was added to ferritin (bovine), which had endogenous iron both in vitro and in vivo. In addition, in that study the estimate of the iron:protein ratio in the isolated ferritin was very high, based on the data in the article, which suggested the presence of denatured ferritin protein/hemosiderin to which some labeled iron added in vitro may have been adsorbed, giving rise to the inappropriately high absorption observed (7). None of these possibilities would have been detected in the protein analyses described in the article by Hunt, because the effect is on the location of the labeled iron in the iron mineral.

The assertion in Hunt’s letter about the plant ferritin mineral in reconstituted horse spleen ferritin is incorrect. We previously compared the mineral in pea ferritin with that in horse spleen ferritin reconstituted to have a plant ferritin mineral composition and found, using EXAFS analysis, that the high phosphate mineral content in horse spleen ferritin was similar to that in natural pea ferritin (8) and distinct from that in animal ferritins; the work was annotated in our recent paper. For Hunt’s assertion to be correct, the nonconserved structural features would have had to dominate the many conserved structural features of ferritin protein in iron absorption. In our recent article, we were merely exploring whether the structure of the plant ferritin mineral influenced iron absorption in humans. Studies currently in progress will explore the influence, if any, of the plant ferritin protein on iron absorption. Hunt misunderstood our statement about soybean cultivars, which simply indicated that soybeans with more ferritin would likely have a greater proportion of the bean iron in ferritin. Under the hydroponic, nonnodulating conditions used in the study described (9), the percentage of bean iron in ferritin was lower than that in field-grown beans. All soybean cultivars have a large fraction of the iron in ferritin when grown under field conditions.

In contrast with the statement in Hunt’s letter, we did not “conclude” that iron from ferritin or ferrous sulfate follow different metabolic pathways after absorption, we merely suggested this as a possible explanation for the differences observed. Further molecular studies at a cellular level are needed to verify this, but our own preliminary data from Caco-2 cells (presented at Experimental Biology 2004) support such a scenario. We did not intend to state that the retention in erythrocytes was greater than that in the whole body. We used the conventional approach for estimating whole-body retention from red blood cell (RBC) incorporation, ie, assuming 80% incorporation and calculating whole-body retention from blood volume estimated from body weight. This was stated in the text as the well-known “RBC incorporation method for estimating iron absorption” rather than in explicated detail in the Methods, which, unfortunately, may have caused confusion. We obtained a higher absorption using this method than when using whole-body counting. In Hunt’s previous work (10), a lower value was obtained with the RBC method (calculated from Figure 1 in reference 10) than with whole-body counting. These differences between the 2 methods for determining iron absorption may be related to differences in the geometry of the counter when determining radioactivity from a point source (blood) rather than from a whole body. However, in our study, the same method was used in the 2 groups of subjects. We observed a difference for ferritin iron and ferrous sulfate, which suggested a difference in metabolic handling of the 2 types of iron. Further studies are needed to explore this in more detail.

To summarize, we showed an efficient absorption (20–25%) of ferritin iron in several studies using different analytic methods to
measure iron incorporation. We avoided the problem of equilibration of unlabeled, solid, mineral iron in ferritin with added labeled iron in 3 ways: 1) using no label (5, 2) using soybean plants to which the label was added before seed ferritin formation (9, 11), and 3) using ferritin from which iron was removed before reconstituting the mineral with labeled iron (1). Earlier, we suggested that both the iron status of the subjects and the labeling method may have contributed to the apparent inconsistencies among the various studies (5, 9). Now it appears more likely that the apparent inconsistencies among previous studies depend most heavily on poor equilibration of labeled iron with endogenous, solid, mineral ferritin in vitro or in vivo if the label is added late in bean development, because the women in our recent study had normal iron status (1).

There was no conflict of interest.

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Hormonal and lifestyle determinants of appendicular skeletal muscle mass in men: the MINOS Study

Dear Sir:

The recent article by Szulc et al (1) identified several lifestyle and hormonal factors as potential determinants of sarcopenia in a large sample of men aged 45–85 y. Appendicular skeletal muscle mass, corrected for body size, displayed a notable age-related decline after the age of 60 y, and routine physical activity both at work and during leisure time were shown to positively affect arm and leg muscle masses, independently of age. Importantly, this investigation indicated that relatively nonstructured, low-to-moderate intensity activities—including bicycling, walking, gardening, and housework—can help to maintain muscle mass in elderly men.

It is interesting to note that we came to a similar conclusion (2) in regard to healthy white postmenopausal women of a comparable age range (54–76 y). A curvilinear relation between muscle mass and age was found, with an accelerated decline after 60–65 y. Those women who routinely engaged in work, household, or leisure activity with a combined energy expenditure of >5 MJ per fortnight had markedly better muscle mass indexes than their less active counterparts; this effect was evident throughout the age range studied. Moreover, as noted by Szulc et al from self-reported data in their male cohort (1), interviews with these women showed the most common leisure-time activities undertaken (walking, dancing, floor exercises, gardening, swimming, and tennis) to be relatively nonstructured and moderate in intensity (2).

Although it must be acknowledged that some training and observational studies have failed to confirm a positive effect of these predominantly aerobic types of activity on mass (3, 4), accumulating evidence supports a role for these popular activities in the maintenance of muscle and the provision of other metabolic benefits, including a reduction of abdominal fat, in both men and women (5). Such activities, therefore, may be useful in preventing sarcopenic obesity, which has been shown to be significantly associated with decreased functional status and increased disability and falls in the elderly (6). The follow-up reports of cohorts such as the MINOS Study subjects should be invaluable in further quantifying these important, practical aspects of the complex relation between physical activity and sarcopenia.

There were no conflicts of interest.

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Erratum


On page 1014, Table 3, the mean daily intakes of olive oil by men and women were incorrect, although there was no error in the corresponding SDs. The correct mean (±SD) intakes (in g) were 53.6 ± 24.0 for men and 46.4 ± 21.8 for women. A programming error was responsible for this overestimation.

The partial regression coefficients of systolic and diastolic blood pressure on SD increments in olive oil intake and the corresponding 95% CIs and P values (reported in Tables 4 and 5 and throughout the text) were unaffected by this error.