The effect of red and white wines on nonheme-iron absorption in humans[1–3]

James D Cook, Manju B Reddy, and Richard F Hurrell

ABSTRACT The effect of the phenolic compounds in wine was examined in this study by performing radioiron-absorption measurements from extrinsically labeled test meals in 33 human subjects. In four separate studies we observed that absorption was 2- to 3-fold higher from white wine containing a low concentration of polyphenols than from two red wines containing a 10-fold higher concentration of polyphenols. The interaction between the polyphenols and alcohol in wine was evaluated by reducing the alcohol content of the wines by ≈90%. When the alcohol concentration was reduced, there was a significant 28% decrease in nonheme-iron absorption with red wine but no effect with white wine. The inhibitory effect of red wines with reduced alcohol content was about twofold greater when they were consumed with a small bread roll than when taken without food. Our findings indicate that the inhibitory effect of phenolic compounds in red wine is unlikely to affect iron balance significantly. *Am J Clin Nutr* 1995;61:800–4.

KEY WORDS Nonheme-iron absorption, polyphenols, alcohol, wine

Introduction

The effect of wine consumption on the iron status of humans has been debated for many years. It was originally suggested that excessive wine intake was the primary cause of the iron-loading disorder hereditary hemochromatosis. MacDonald (1), who was the main proponent of this theory, estimated that the large quantities of wine consumed by chronic alcoholics could more than double their daily intake of iron and thereby account for the modest daily increment in absorbed iron that eventually produces the clinical manifestations of this disease. It is now known that hereditary hemochromatosis is due to the inheritance of an iron-loading gene (2) and that the excessive accumulation of body iron occurs in patients consuming a normal amount of dietary iron.

More recent studies indicate that the iron in wine is not well absorbed and that red wine in particular impairs the absorption of nonheme iron. Bezwoda et al (3) reported that the absorption of iron added to red wine was only 20% of that of a solution of 7% alcohol. On the basis of a series of in vitro studies and the demonstration that a significant increase in iron absorption occurred when 80% of the polyphenols was removed, these workers concluded that the low availability of the iron in red wine was due to the binding of iron to polyphenols. It is possible that this inhibitory effect of polyphenols can increase the risk of iron deficiency or prevent the progressive accumulation of iron stores with age in those consuming red wine regularly.

The present investigation was undertaken to further evaluate iron absorption from wine by performing radioiron-absorption tests in adult subjects. Iron absorption was measured from wine alone and from test meals containing wine and an unfortified dinner roll. In most studies, iron absorption from a solution of water served as a control. We compared absorption from three wines that varied widely in their polyphenol content and evaluated the effect on iron absorption of extensively reducing the alcohol content of these wines.

Subjects and methods

Subjects

Iron absorption was measured in 33 volunteer subjects aged 21–33 y. The total group included 17 males and 16 females. All subjects were in good health and denied a history of disorders known to influence the gastrointestinal absorption of iron. Serum ferritin determinations ranged from 5 to 158 μg/L, indicating a wide variation in iron status. Five of the subjects, two men and three women, were iron-deficient as defined by a serum ferritin concentration ≤ 12 μg/L. Iron deficiency in the two male volunteers was due to regular blood donations. Written, informed consent was obtained from each volunteer before the investigation and all experimental procedures were approved by the Human Subjects Committee at the University of Kansas Medical Center.

Iron-absorption measurements

Four separate iron-absorption measurements were performed in each subject by using double radioiron tracers administered sequentially. All meals were administered between 0700 and 0900 after an overnight fast and nothing but water was allowed for a further 3 h. The test meals were labeled by the extrinsic

[1] From the Division of Hematology, Department of Medicine, Kansas University Medical Center, Kansas City, and the Laboratory for Human Nutrition, Swiss Federal Institute of Technology, Ruschlikon, Switzerland.

[2] Supported by NIH grant DK39246.

[3] Address reprint requests to JD Cook, Division of Hematology, Kansas University Medical Center, 3901 Rainbow Boulevard, Kansas City, KS 66160–7402.

Received June 2, 1994.

Accepted for publication November 3, 1994.

tag method as previously described by adding either 37 kBq $^{59}FeCl_3$ or 111 kBq $^{55}FeCl_3$ to a 1-mL solution containing 0.1 mg Fe as $FeCl_3$ in 0.01 mol HCl/L (4).

On the day preceding administration of the first test meal, 30 mL blood was obtained from each subject for measurement of packed cell volume, serum ferritin (5), and background radioactivity. The first and second test meals, labeled with ^{59}Fe and ^{55}Fe, respectively, were fed on days 2 and 3 of the study. Fourteen days after administration of the second of these meals (day 16), 30 mL blood was drawn for measurement of incorporated red blood cell radioactivity. A third and fourth test meal tagged with separate radioiron labels were fed on days 16 and 17 and a final blood sample was obtained on day 31 to determine the increase in red blood cell radioactivity. Measurements of blood radioactivity were performed on duplicate 10-mL samples of whole blood by a modification of the method of Eakins and Brown (6). Percentage absorption was calculated on the basis of the blood volume estimated from height and weight (7,8) and an assumed red cell incorporation for absorbed radioactivity of 80% (9).

Composition of test meals

One white wine and two red wines were selected for evaluation on the basis of their polyphenol content. The white wine contained a low concentration of polyphenols (0.19 g/L) whereas the two red wines, a pinot noir and an aramon, contained 2.98 and 1.95 g total polyphenols/L, respectively. Batches of all three wines were rendered low in alcohol by distillation under a vacuum of 2500 Pa at 35 °C. The wine was then filtered under nitrogen, bottled, and pasteurized at 65 °C for 20 min. The low-alcohol wines still contained ≈10% of their original alcohol concentration. For organoleptic reasons, equal amounts of glucose and fructose were added to the low-alcohol wines to increase their content of total sugars to 30 g/L. The organic acid and polyphenol contents of the wines was not altered appreciably by the method used to reduce the alcohol content (**Table 1**). The normal and low-alcohol wines were kindly supplied and their chemical composition determined according to the international methods for wine analysis (10) by Bourzeix, Institute des Produits de la Vigne, Narbonne, France. Iron was determined by atomic-absorption spectroscopy after dry-ashing.

Four separate studies were performed with eight or nine subjects in each group. In all but one study, 120 mL wine was fed with a 70-g dinner roll prepared with nonenriched flour and that contained 0.54 mg Fe. Thus, the total iron content of the test meals ranged from 0.74 to 1.0 mg Fe when the meal contained a roll and from 0.22 to 0.64 mg Fe when the wine was served alone (study 4). In all but study 4, a control test meal containing deionized water rather than wine was administered, and the iron content of the meal in this case was 0.54 mg. The effect of differences in the iron status of the subjects within and between studies could be eliminated by relating absorption of the test meals containing wine to the meals containing the water control. All test meals that contained a roll were labeled by pipetting the extrinsic tag onto the roll.

Statistical methods

Percentage absorption values were converted to logarithms before performing statistical analysis and the results were retransformed to antilogarithms to recover the original units (11). Paired *t* tests were used to compare absorption from selected test meals within each study by determining whether the mean log absorption ratios differed significantly from zero. The ratios of absorption from red and white wines in different studies were assessed by analysis of variance (ANOVA) and the differences in these ratios were tested by Scheffe's post hoc test (ABSTAT; Anderson-Bell Corp, Parker, CO).

Results

In study 1, absorption from bread meals containing the alcoholic wines was compared with the test meal containing water (**Table 2**). Iron absorption means from the meals containing red wine were less than one-half of those observed from

TABLE 1
Composition of wines

	White wine		Aramon		Pinot noir	
	Normal alcohol	Low alcohol	Normal alcohol	Low alcohol	Normal alcohol	Low alcohol
Alcohol (g/L)	105.0	13.2	81.3	9.8	93.9	8.7
Total sugars (g/L)	1.7	29	2.2	30	2.6	30
Acidity						
pH	3.4	3.3	3.7	3.6	3.6	3.5
Tartaric acid (g/L)	1.8	1.6	2.2	2.2	2.9	3.2
Malic acid (g/L)	1.9	1.7	0	0	0	0
Lactic acid (g/L)	0	0	1.4	1.3	1.0	0.9
Citric acid (g/L)	0.1	0.1	0	0	0	0
Metals (mg/L)						
Calcium	78	98	62	96	80	104
Iron	1.8	1.8	3.8	3.4	1.7	1.9
Polyphenols (g/L)						
Total (as gallic acid)	0.19	0.22	1.95	1.83	2.98	2.97
Catechin	—	—	0.062	0.058	0.116	0.134
Epicatechin	—	—	0.024	0.017	0.068	0.053
Proanthocyanidols	—	—	0.123	0.088	0.307	0.271
Anthocyanins	—	—	0.232	0.206	0.340	0.346

TABLE 2
Iron absorption from red and white wines with normal and reduced alcohol content

Study and subjects' age	Packed cell volume	Serum ferritin[1]	Meals	Iron absorption[1]	Absorption ratios[1] vs Meal B	vs Meal C	vs Meal D
	%	μg/L		% of dose			
1 (n = 7M, 1F; 24 y)			A (bread, aramon				
	44	49	wine)	5.05 (3.63–7.04)	1.25 (1.14–1.37)	0.44[2] (0.37–0.52)	0.32[2] (0.25–0.41)
			B (bread, pinot noir				
		(36–66)	wine)	4.05 (2.79–5.87)	—	0.35[2] (0.28–0.45)	0.25[2] (0.19–0.35)
			C (bread, white wine)	11.43 (8.70–15.02)	—	—	0.72 (0.61–0.86)
			D (bread, water)	15.89 (12.81–19.70)	—	—	—
2 (n = 2M, 6F; 26 y)			A (bread, low-alcohol				
	42	29	aramon)	3.31 (1.91–5.72)	1.07 (0.93–1.23)	0.28[2] (0.22–0.39)	0.23[2] (0.18–0.31)
			B (bread, low-alcohol				
		(20–42)	pinot noir)	3.08 (1.81–5.25)	—	0.26[2] (0.21–0.34)	0.22[4] (0.17–0.28)
			C (bread, low-alcohol				
			white wine)	11.66 (7.87–17.28)	—	—	0.83 (0.73–0.94)
			D (bread, water)	14.12 (10.17–19.61)	—	—	—
3 (n = 7M, 1F; 24 y)			A (bread, pinot noir				
	44	37	wine)	3.32 (2.30–4.80)	1.39[4] (1.26–1.53)	0.35[3] (0.30–0.41)	0.32[3] (0.26–0.39)
			B (bread, low-alcohol				
		(27–53)	pinot noir)	2.39 (1.60–3.56)	—	0.25[3] (0.31–0.31)	0.23[3] (0.18–0.29)
			C (bread, white wine)	9.43 (6.82–13.03)	—	—	0.91 (0.83–1.0)
			D (bread, low-alcohol				
			white wine)	10.38 (7.45–14.46)	—	—	—
4 (n = 2M, 6F; 23 y)			A (low-alcohol aramon				
	42	30	wine)	21.75 (13.71–34.52)	1.07 (0.95–1.20)	0.51[5] (0.40–0.64)	0.80 (0.50–1.28)
			B (low-alcohol pinot				
		(21–42)	noir)	20.31 (13.03–31.65)	—	0.48[4] (0.39–0.58)	0.75 (0.50–1.20)
			C (low-alcohol white				
			wine)	42.71 (32.79–55.63)	—	—	1.57 (1.15–2.15)
			D (water)	27.16 (19.68–37.49)	—	—	—

[1] Geometric mean; range in parentheses.
[2] $P < 0.005$.
[3] $P < 0.001$.
[4] $P < 0.01$.
[5] $P < 0.05$.

the meals containing white wine or water; the mean absorption ratios of 0.32 and 0.25 for red wine/water control, respectively, were both highly significant. The mean absorption of 11.43% observed with white wine was also significantly higher than with both red wines, whereas iron absorption with white wine did not differ significantly from water, indicating that white wine has a negligible effect on nonheme-iron absorption. Mean absorption from the aramon red wine containing the lower content of polyphenols was 25% higher than that of the pinot noir red wine, 5.05% and 4.05%, respectively, but the difference was not statistically significant.

The design of study 2 was identical to that of the previous study, except that low-alcohol wines were used. The findings were similar in that iron absorption from meals containing red wine was significantly lower than that from both meals with water and those with white wine. As in study 1, absorption with white wine was not significantly different from that with water. Absorption means with meals containing the red wines were not significantly different.

In study 3, absorption from the normal and low-alcohol wines was compared directly. In the first pair of absorption tests, mean absorption with normal and low-alcohol pinot noir averaged 3.32% and 2.39%, respectively; the mean absorption ratio for normal and low-alcohol wines of 1.39 was statistically significant. On the other hand, the mean absorption values with normal and low-alcohol white wines of 9.43% and 10.38%, respectively, did not differ significantly (P = 0.36).

The first three studies were designed to examine the interaction between wine and a small amount of food. In study 4, iron absorption from wine alone was examined by using the low-alcohol products. Absorption values were dramatically higher. Mean absorption values of 21.75% and 20.31% with the red wines did not differ from absorption with water containing 0.1 mg Fe as the extrinsic tag ($P > 0.50$). The sharply higher mean absorption of 42.71% observed with white wine was significantly different from the values observed with both red wines but not from the water control.

A more detailed evaluation of the effect of the polyphenol content of wine was obtained by comparing the absorption ratios for red and white wines in the three studies in which all three wines were tested (studies 1, 2, and 4). The mean ratios obtained with pinot noir were consistently lower than with aramon, in keeping with the modestly higher polyphenol content of the pinot noir (**Figure 1**). However, two-way ANOVA did not indicate a significant difference in the ratios observed with the two red wines when all three studies were evaluated

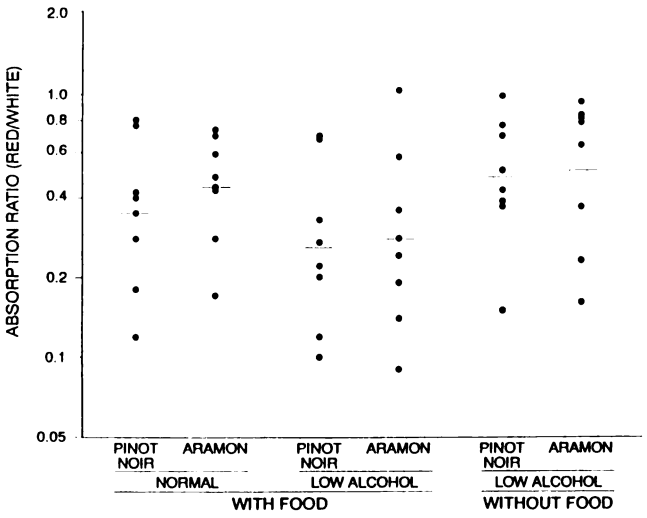

FIGURE 1. Iron-absorption ratios for red and white wines. The horizontal bars depict geometric means. For pinot noir and aramon, these were 0.35 and 0.44, respectively, when normal wines were given with food (pooled mean 0.39); 0.26 and 0.28 when low-alcohol wines were given with food (pooled mean 0.27); and 0.48 and 0.51 when given without food (pooled mean 0.49).

(F = 0.389, P = 0.53). Consequently, the ratios observed for the two wines in each study were pooled. A significant difference in the pooled ratio for the two red wines and the white wine between the three studies was observed (F = 3.42, P = 0.04). The mean ratio of 0.27 for red and white wines when the low-alcohol wines were given with food was significantly lower than the mean of 0.49 observed when the low-alcohol wines were given without food (P = 0.037), indicating perhaps that the polyphenols in red wine interact with a component of the bread roll to increase their inhibiting effect. This interaction is lessened in the presence of alcohol because the mean ratio for red and white wines of 0.39 in study 1 did not differ from the ratios observed with low-alcohol wine taken either with or without food.

Discussion

The potential influence on iron status of a dietary food or beverage that is consumed regularly depends on its iron content, the availability of this iron, and its effect on iron absorption from foods with which it is consumed. The iron content of wine varies widely depending to some extent on the iron content of the soil in which the grapes are grown. The manufacturing practices are of greater importance (12) because the iron content of wine is increased by contaminating iron from the machinery and installations with which it has contact during processing. In general, rosé wines, with their higher acidity and sulfur dioxide content, are more corrosive and have higher iron contents (13). In one study in which the iron contents of nearly 200 wines imported into Sweden were measured, the iron concentration ranged from 3 to 20 mg Fe/L for red wines and from 1 to 18 mg Fe/L for white wines (14). The overall means were 7.2 and 5.2 mg/L for red and white wines, respectively. Similarly, the mean iron content of 49 wines imported into Finland was reported as 7.4 mg/L for red wines (range 3.6–15.4 mg/L), 5.9 mg/L for white wines (range 1.7–5.9 mg/L), and 9.6 mg/L for rosé wines (range 4.6–11.6 mg/L) (15). Other workers have reported the mean iron concentration of Italian wines to be 5.9 mg/L (range 0.9–10.2 mg/L) (12), of Spanish wines to be 4.8 mg/L (range 2.4–10.2 mg/L) (13), and of California wines to be 3.3 mg/L (range 0.3–16.1 mg/L) (16). The relatively low iron content of the wines used in the present study (1.8–3.8 mg/L, Table 1) reflects the fact that they were produced in a wine research center and not in a commercial winery. Wine is therefore a fairly good iron source with a mean iron content of ≈5 mg/L. In countries such as France where the average adult daily intake of wine is 300 mL (17), this represents 1–2 mg Fe/d, or 5–10% of daily iron intake.

The intrinsic bioavailability of the iron in the three wines evaluated in the present study was relatively high when the wine was consumed without food (Table 2). Iron absorption from the three wines did not differ significantly from that from water although absorption from white wine was actually 50% higher than that from water. This difference presumably reflects a facilitating effect on iron absorption resulting from its organic acid content (18). Absorption from the two red wines with higher polyphenol content was significantly lower than that from white wine by ≈50%. This difference is remarkably similar to that reported by Bezwoda et al (3), who found an average absorption of 4.4% from red wine and 10.4% from white wine, both fortified with 3 mg Fe as $FeSO_4$.

Because wine is usually consumed with food, a more relevant issue with respect to the effect of wine consumption on iron balance is its influence on dietary nonheme-iron absorption. In the present study we observed a doubling of the inhibitory effect of the polyphenols in low-alcohol red wines when consumed with a bread roll (Table 2, Figure 1). It is likely that the greater inhibitory effect of polyphenols in the presence of food is due to the formation of an insoluble complex of iron, polyphenols, and protein digestion products within the intestinal lumen, which are less soluble than the iron-polyphenol complex in wine alone. The interaction between polyphenols and protein is well known. For example, vegetable tannins are defined as polyphenols, which have the property of precipitating protein (19). A similar observation has been made with the in vitro dialyzable iron method. Brown et al (20) showed that tea polyphenols alone bound to iron and that the complex passed the dialysis membrane. However, when a cereal-based milk meal was present, the tea polyphenol-iron complex bound to the partially digested meal components and no longer passed through the dialysis membrane. Note that other workers have not demonstrated an inhibiting effect of wine on iron absorption from a larger hamburger meal (14). Perhaps the content of known enhancers of iron absorption in this meal that contained muscle tissue and ascorbic acid offset the inhibitory properties of the wine polyphenols. The difference could also be explained by the larger size of the meal, which may have diluted the inhibitory effects of red wine polyphenols.

Another important constituent of wine that may influence dietary iron absorption is its alcohol content. There have been several prior studies of the effect of alcohol on iron absorption, with variable findings. One of the earlier reports examined the influence of alcohol on the absorption of inorganic iron (21). Alcohol had no influence on the absorption of ferrous ascorbate but increased the absorption of ferric chloride more than five-

fold. Because this stimulating effect did not occur in patients with achlorhydria, it was proposed that alcohol enhances iron absorption by stimulating gastric acid secretion. Hallberg and Rossander (14) demonstrated a modest but significant 23% increase in nonheme-iron absorption from a hamburger meal when consumed with distilled alcohol but not with beer or wine. In the present study, the influence of alcohol contained in wine was evaluated more directly by studying iron absorption from wine before and after a 90% reduction in alcohol content. A significant enhancing effect of alcohol was observed with red but not with white wine although the difference was relatively small (study 3, Table 2), indicating that the alcohol in wine has a rather limited influence on nonheme-iron absorption. The effect of alcohol presumably also depends on the quantity of food consumed, which itself will influence gastric secretion. The modest 20% difference observed in the present study is unlikely to persist with larger meals.

A relatively low incidence of coronary heart disease in wine drinkers in France consuming a high-fat diet was recently reported (22). Of interest is a recent report that men with higher iron stores, as reflected by serum ferritin determinations, have a sharply increased frequency of myocardial infarction (23). It is possible that the phenolic substances in red wine produce an inhibition of iron absorption and thereby protect against the development of increasing iron stores with age. The modest inhibition of iron absorption from red wine observed in the present study does not support this hypothesis. If there is indeed an association between red wine intake and ischemic heart disease, it is more likely due to the antioxidant properties of red wine polyphenols (22).

References

1. MacDonald RA. Idiopathic hemochromatosis: genetic or acquired? Arch Intern Med 1963;112:184–90.
2. Simon M, Le Mignon L, Fauchet R, et al. A study of 609 HLA haplotypes marking for the hemochromatosis gene: (1) mapping of the gene near the HLA-A locus and characters required to define a heterozygous population and (2) hypothesis concerning the underlying cause of hemochromatosis-HLA association. Am J Hum Genet 1987; 41:89–105.
3. Bezwoda WR, Torrance JD, Bothwell TH, MacPhail AP, Graham B, Mills W. Iron absorption from red and white wines. Scand J Haematol 1985;34:121–7.
4. Cook JD, Layrisse M, Martinez-Torres C, Walker R, Monsen E, Finch CA. Food iron absorption measured by an extrinsic tag. J Clin Invest 1972;51:805–15.
5. Flowers CA, Kuizon M, Beard JL, Skikne BS, Covell AM, Cook JD. A serum ferritin assay for prevalence studies of iron deficiency. Am J Hematol 1986;23:141–51.
6. Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Radiat Isot 1966;17:391–7.
7. Wennesland R, Brown E, Hopper J Jr, et al. Red cell, plasma and blood volume in healthy men measured by radiochromium (Cr51) cell tagging and hematocrit: influence of age, somatotype and habits of physical activity on variance after regression of volumes to height and weight combined. J Clin Invest 1959;38:1065–77.
8. Brown E, Hopper J Jr, Hodges JL Jr, Bradley B, Wennesland R, Yamauchi H. Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. J Clin Invest 1962;41:2182–90.
9. Hosain F, Marsaglia G, Finch CA. Blood ferrokinetics in normal man. J Clin Invest 1967;46:1–9.
10. International Office of Vine and Wine. (Office International de la Vigne et du Vin.) Review of international methods of analysis of wines and derivatives. (Recueil des methodes internationales d'analyse des vins et des mouts.) Paris: OIV, 1990.
11. Cook JD, Layrisse M, Finch CA. The measurement of iron absorption. Blood 1969;33:421–9.
12. Interesse FS, Lamparelli F, Allogio V. Mineral content of some Southern Italian wines. Z Lebensm Unters Forsch 1984;178:272–8.
13. Gonzales MJ, Para MCM, Aguilar MV. Determination of Fe, Cu, Zn, Mn and Pb in D.O.C. Mentrida wines. Z Lebensm Unters Forsch 1988;187:325–9.
14. Hallberg L, Rossander L. Effect of different drinks on the absorption of non-heme iron from composite meals. Hum Nutr Appl Nutr 1982; 36:116–23.
15. Harju K, Ronkainen P. Analysis of wines for metals using atomic absorption spectrophotometry. Z Lebensm Unters Forsch 1980;170: 445–8.
16. Ough CS, Crowell EA, Benz J. Metal content of California wines. J Food Sci 1982;47:825–8.
17. Darret G, Couzy F, Antoine JM, Magliola C, Mareschi JP. Estimation of minerals and trace elements provided by beverages for the adult in France. Ann Nutr Metab 1986;30:335–44.
18. Gillooly M, Bothwell TH, Torrance JD. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br J Nutr 1983;49:331–42.
19. Gupta RK, Haslam E. Vegetable tannins— structure and biosynthesis. Ottawa: IRDC, 1980.
20. Brown RC, Klein A, Simmons WK, Hurrell RF. The influence of Jamaican herb teas and other polyphenol-containing beverages on iron absorption in the rat. Nutr Res 1990;10:343–53.
21. Charlton RW, Jacobs P, Seftel H, Bothwell TH. Effect of alcohol on iron absorption. Br Med J 1964;2:1427–9.
22. Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 1993;341:454–7.
23. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. Circulation 1992;86: 803–11.