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Alcohol and high-density-lipoprotein cholesterol: a randomized controlled trial

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1. A randomized controlled trial of cross-over design was set up to examine the effect of alcohol on blood lipids and certain haematological variates relevant to ischaemic heart disease.

2. One hundred subjects drank some alcohol for 4 weeks (mean intake 18·4 g/d) and abstained totally for 4 weeks, the order of these periods being randomized.

3. Alcohol appeared to produce a rise of 7% in serum high-density-lipoprotein (HDL) cholesterol, probably due to a rise in the HDL_2 subfraction.

4. No significant change was detected in plasma fibrinogen or the other haematological indices.

5. These results are consistent with the hypothesis that a moderate intake of alcohol confers some protection against heart disease.

There is a body of evidence suggesting that persons who take small quantities of alcohol have a lower mortality from ischaemic heart disease (IHD) than either heavy drinkers or non-drinkers (Hennekens *et al.* 1978; Yano *et al.* 1978; St Leger *et al.* 1979; Kozarareric *et al.* 1980; Klatsky *et al.* 1981; Marmot *et al.* 1981). Several studies have shown an association between alcohol intake and serum high-density-lipoprotein (HDL) cholesterol (Hulley & Gordon, 1981), which is associated with a lower risk of IHD. A randomized controlled trial was set up to test these associations, together with the association between alcohol and plasma fibrinogen found in a cross-sectional study (Yarnell *et al.* 1983).

SUBJECTS AND METHODS

The subjects were recruited from amongst the office workers of two local government authorities together with employees of the Medical Research Council. Diabetics were excluded from the study, and so were persons taking contraceptives or hypertensive drugs. The subjects were randomly divided into two groups, one of which was asked to abstain totally from alcohol for 4 weeks while those in the other group were asked to take at least seven alcoholic drinks weekly. One drink constituted a half-pint (284 ml) of beer or cider, one glass of wine or a measure of sherry or spirits, providing 7–9 g alcohol. Details were recorded by the latter subjects of the type, number and quantity of every alcoholic drink taken during this period. After 4 weeks a fasting blood sample was taken from each subject for haematological and lipid analyses. The two groups were then transposed for a further period of 4 weeks, and the blood measurements repeated. The subjects were weighed before entering the trial and at the end of each period in it. No objective measure of compliance was used, but the subjects were carefully selected and understood the importance of adhering to the protocol.

Lipoproteins were isolated from fasting plasma by a combination of precipitation with sodium phosphotungstate–magnesium chloride to isolate HDL (Lopes-Virella *et al.* 1977), and ultracentrifugation using a Beckman Airfuge (Eyre *et al.* 1981) to isolate very-low-density lipoprotein (VLDL) and HDL subfractions. Plasma was ultracentrifuged at 132000 ***g*** for 3·5 h in the Beckman Airfuge. The resultant upper layer of VLDL was sliced off using a custom-built tube slicer and retained for further analysis. The HDL supernatant fraction following precipitation was adjusted to a density of 1·125 g/ml with potassium bromide–sodium chloride solution and ultracentrifuged for 3·5 h. The upper layer of HDL_2 was sliced off and discarded and the residual HDL_3 retained.

Cholesterol and triglyceride levels were measured in plasma, VLDL, HDL and HDL_3 (cholesterol only) by standard enzymic methods using commercially available kits (Boehringer Corporation Ltd). Low-density lipoprotein (LDL) and HDL_2 levels were obtained by difference.

Plasma fibrinogen was measured by the method of Thorp *et al.* (1967). Plasma viscosity was determined using a Coulter Viscometer (Harkness type).

Statistical tests for treatment effects, period effects and treatment–period interactions were performed using the large-sample test statistics described by Hills & Armitage (1979).

RESULTS

One hundred and twelve subjects were recruited, of whom 100 completed the study, comprising forty-eight men and fifty-two women. Their mean ages were 37·7 years (range 20–56) and 40·5 years (range 19–60) respectively. Fifty-two were randomly allocated to take alcohol first (group A) followed by a period of abstention, while 48 abstained first (group B). Their mean daily alcohol intakes during the appropriate periods were 19·0 (SD 14·0) g and 17·8 (SD 7·7) g respectively, the lowest individual mean daily intake being 5·6 g/d. The usual alcohol intake was available for fifty-eight subjects, ten of whom habitually took less than two drinks weekly, forty-one two to nineteen drinks weekly, and seven twenty or more drinks weekly.

Table 1 shows the mean serum lipid levels of the two groups after each of the two periods. The distributions of several of the subfractions are highly skewed, especially for VLDL triglyceride, owing to a few subjects with high values. There was a tendency for total cholesterol, total triglyceride and VLDL triglyceride to rise from the first test to the second irrespective of whether alcohol had been taken; this was probably a seasonal effect, since these levels were found to rise from May to December, during the period when most subjects took part. There was, however, no interaction effect between alcohol and time-period for these or any other variables, i.e. the effects (if any) of alcohol and time-period appeared to be independent of each other. There was one subject for whom no lipid result was obtained on one occasion, so the total number is shown as ninety-nine. There were a few other subjects whose subfractions of cholesterol and triglyceride were not measured on one or other occasion; this explains the apparent discrepancies between the sum of the subfractions and the total HDL cholesterol and triglyceride concentrations in group B.

Table 2 shows the mean differences in lipid concentrations between the values obtained when the subjects had been drinking and those when they had been abstaining. Statistically significant differences were found in HDL cholesterol, HDL as a percentage of total cholesterol, and HDL triglyceride concentrations. In each case the values after alcohol were higher than those after abstaining, by 7, 5 and 15% overall respectively in the two groups combined. Although the rise in HDL_2 did not quite achieve statistical significance it was both larger (12%) and more consistent than the change in HDL_3 cholesterol (1%), which showed a rise with alcohol in one group and a fall in the other. For HDL_2 cholesterol, VLDL

Table 1. *Serum lipid concentrations (mmol/l) after alcohol or abstinence from alcohol in ninety-nine subjects*

(Mean values and standard errors)

	Group A (alcohol first)						Group B (alcohol second)					
	After alcohol			After abstaining			After abstaining			After alcohol		
	n	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
Cholesterol												
Total	51	5·45	0·16	51	5·57	0·18	48	5·26	0·15	48	5·45	0·14
VLDL	51	0·45	0·05	51	0·44	0·06	48	0·40	0·04	47	0·43	0·04
LDL	51	3·69	0·16	51	3·90	0·18	48	3·67	0·13	47	3·71	0·14
HDL	51	1·32	0·04	51	1·23	0·06	48	1·20	0·05	48	1·29	0·05
HDL_2	51	0·57	0·04	49	0·55	0·06	45	0·47	0·04	47	0·57	0·04
HDL_3	51	0·75	0·03	49	0·70	0·03	45	0·72	0·03	47	0·70	0·03
Triglyceride												
Total	51	1·12	0·13	51	1·21	0·12	48	0·96	0·06	48	1·12	0·08
VLDL	50	0·36	0·10	50	0·43	0·09	48	0·27	0·03	47	0·37	0·07
LDL	50	0·43	0·04	50	0·47	0·04	48	0·40	0·04	47	0·41	0·04
HDL	51	0·34	0·02	51	0·32	0·02	48	0·30	0·01	48	0·38	0·03

VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HDL_2, HDL_3, HDL subfractions.

Table 2. *Differences (mmol/l) between lipid values for ninety-nine subjects when taking and abstaining from alcohol*

(Mean values and standard errors)

	Value on alcohol minus value when abstaining						
	Group A (alcohol first)			Group B (alcohol second)			
		Difference			Difference		Test statistic for alcohol
	n	Mean	SE	*n*	Mean	SE	
Cholesterol							
Total	51	−0·12	0·09	48	0·19	0·09	0·52
VLDL	51	0·01	0·03	47	0·04	0·05	0·79
LDL	51	−0·21	0·10	47	0·05	0·09	−1·16
HDL	51	0·08	0·05	48	0·09	0·04	2·75*
HDL_2	49	0·03	0·06	44	0·09	0·04	1·77
HDL_3	49	0·05	0·04	44	−0·03	0·03	0·49
HDL(%)	51	1·64	1·07	48	0·95	0·60	2·11*
Triglyceride							
Total	51	−0·09	0·06	48	0·16	0·07	0·77
VLDL	49	−0·07	0·03	47	0·11	0·07	0·55
LDL	49	−0·03	0·04	47	0·02	0·05	−0·20
HDL	51	0·01	0·03	48	0·08	0·04	2·12*

VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HDL_2, HDL_3, HDL subfractions.

* $P < 0·05$.

Table 3. *Values of haematological indices after alcohol or abstinence from alcohol in ninety-nine subjects*

(Mean values and standard errors)

	Group A						Group B					
	After alcohol			After abstaining			After abstaining			After alcohol		
	n	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*	Mean	SE
Haemoglobin (g/l)	52	143·9	1·5	51	144·3	1·6	48	142·4	1·5	48	142·7	1·7
Fibrinogen (g/l)	52	3·49	0·11	50	3·38	0·11	48	3·71	0·12	48	3·34	0·11
Viscosity (cP)	52	1·67	0·01	51	1·66	0·01	48	1·66	0·01	48	1·66	0·01

triglyceride and HDL triglyceride the differences had skewed distributions which were not restored to normality by logarithmic transformation of the data. A non-parametric test (the Wilcoxon two-sample rank test) was therefore performed for these lipids. The treatment effect of alcohol on HDL_2 cholesterol was highly significant ($P < 0{\cdot}01$) using this test; for VLDL and HDL triglyceride the changes were not significant ($P > 0{\cdot}05$). When a similar test was performed for HDL cholesterol the treatment effect was highly significant ($P < 0{\cdot}001$).

Table 3 shows the mean values of certain haematological indices. The total number of subjects is again shown as ninety-nine since haematological results were not available for one subject on one occasion. The differences in paired values of haemoglobin, plasma fibrinogen and plasma viscosity were examined but no statistically significant change occurred; these results are therefore not presented in detail.

The mean weights of the two groups were examined initially and after each period in the trial. The initial weights of the groups were similar (71·2 kg in group A, 70·1 kg in group B); analysis of the individual data showed no significant difference attributable to alcohol or time-period in the trial. Further details are therefore not presented here.

DISCUSSION

People who drink small quantities of alcohol seem to have a lower mortality from all causes and particularly from IHD in comparison with other people (Hennekens *et al.* 1978; Yano *et al.* 1978; Kozarareric *et al.* 1980; Klatsky *et al.* 1981; Marmot *et al.* 1981). The risks of heavy drinking are of course considerable, but total abstainers appear to incur some extra risk too: in one cohort study their total mortality was about 50% higher than that of moderate drinkers, apparently due to increased coronary disease mortality (Klatsky *et al.* 1981). To some extent the relation with total mortality could be due to a tendency for persons in ill health to avoid alcohol in consequence of their disease, but it is difficult to see why this should specifically affect patients with heart disease. It has therefore been suggested that alcohol may confer some protection against IHD.

The concentration of serum HDL cholesterol is inversely related to the risk of myocardial infarction, and it is therefore important to investigate its determinants. Several studies have shown that it is associated with alcohol intake, although whether the association is directly causal is not entirely clear. A few experimental studies have been conducted in non-alcoholic subjects, but all have been small and only one was a randomized trial. Most of these studies have shown a rise in HDL cholesterol in association with alcohol (or a fall in association with abstinence) (Berg & Johansson, 1973; Belfrage *et al.* 1977; Hulley & Gordon, 1981; Thornton *et al.* 1983; Haskell *et al.* 1984); one study showed no effect (Glueck *et al.* 1980).

The largest of these trials, and the only one conducted with randomly allocated controls, involved twenty-four subjects, half of whom drank alcohol for 6 weeks while the other half abstained (Haskell *et al.* 1984). HDL cholesterol fell with abstinence and rose with drinking; these changes seemed to occur in the HDL_3 subfraction rather than the HDL_2 subfraction, which is thought to be the more important with regard to IHD.

The trial reported here involved 100 subjects, each of whom completed 4 weeks on alcohol and 4 weeks of abstinence so as to act as his or her own control. This trial examined the effect of alcohol on blood lipids and also on plasma fibrinogen, which was negatively associated with alcohol intake in a cross-sectional study (Yarnell *et al.* 1983).

The results of the trial suggest that alcohol caused a rise in serum HDL cholesterol, both absolutely and as a percentage of total cholesterol. This was probably due to a rise in the HDL_2 rather than the HDL_3 subfraction. These findings contrast with those in the smaller trial by Haskell *et al.* (1984) where the change occurred in the HDL_3 subfraction. It is important to note that in the Haskell *et al.* (1984) trial the absolute amounts of HDL subfractions were measured, as determined in the analytical ultracentrifuge. This may not reflect accurately, or in parallel, the cholesterol content of the HDL subfractions as we have measured them. There is some evidence, for instance, that determination of HDL subfractions following rate-zonal ultracentrifugation gives different results from the values obtained for HDL subfraction cholesterol (particularly HDL_3 cholesterol) obtained by differential precipitation (Simpson *et al.* 1982). We have used a method similar to that of Miller and his colleagues in their study, which showed a negative association between IHD and HDL_2 cholesterol (Miller *et al.* 1981). No change in plasma fibrinogen or viscosity was attributable to alcohol.

It is theoretically possible that the changes in HDL cholesterol arose not from the alcohol but from other alterations in diet or lifestyle. We do not have accurate details about other aspects of the subject's diets, but they reported that they replaced alcohol mostly by soft drinks during the abstinence period and otherwise kept their diet and lifestyle fairly constant. The available evidence suggests that alcohol is far more likely to raise HDL cholesterol than soft drinks are to lower it. The subjects were mostly light or moderate drinkers by inclination, and the mean amounts taken during the alcohol period correspond to about two drinks daily, so that we are not examining the effect of large intakes. Admittedly a few subjects drank fairly heavily during the alcohol period (whether from a desire to be helpful or for other reasons we did not enquire), but the majority took only moderate amounts. We have no measure of compliance which is independent of our subjects' veracity; should any alcohol have been surreptitiously consumed during the 'abstinence' period the effect of alcohol on HDL cholesterol is likely to be greater than our results suggest.

Thus this trial tends to support the hypothesis, derived from prospective studies, that a little alcohol confers some protection against IHD. A rise in HDL cholesterol, or even in HDL_2 cholesterol, cannot be assumed necessarily to imply protection against IHD, since the associations between these lipids and a reduced risk may not be directly causal. It is difficult to envisage a controlled trial of alcohol intake in which the end-point is myocardial infarction, so the evidence will probably never be complete. Even if it were, the benefits of alcohol would have to be carefully weighed against its considerable risks. We are well aware of the obvious dangers of excessive drinking and have emphatically no wish to see any general increase in the amount of alcohol consumed. But it may perhaps be true that 'a little of what you fancy does you good'.

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