Journal of Epidemiology and Community Health, 1988, **42**, 220-225

Associations of alcohol consumption with plasma high density lipoprotein cholesterol and its major subfractions: The Caerphilly and Speedwell Collaborative Heart Disease Studies

N E MILLER,*[1] C H BOLTON,[2] T M HAYES,[3] D BAINTON,[4] J W G YARNELL,[5] I A BAKER,[4] AND P M SWEETNAM[5]

From the Department of Chemical Pathology & Metabolic Disorders,[1] St. Thomas's Hospital Medical School, London SE1 7EH; Department of Medicine, Bristol Royal Infirmary,[2] Bristol BS2 8HW; Department of Medicine, University Hospital of Wales,[3] Heath Park, Cardiff CF4 4XN; Bristol & Weston Health Authority,[4] 10 Marlborough Street, Bristol BS1 3NP; and MRC Epidemiology Unit,[5] 4 Richmond Road, Cardiff CF2 3AS

J Epidemiol Community Health: first published as 10.1136/jech.42.3.220 on 1 September 1988. Downloaded from http://jech.bmj.com/ on May 29, 2023 by guest. Protected by copyright.

SUMMARY In surveys of 4860 middle-aged men in Caerphilly (South Wales) and Speedwell (Bristol) alcohol consumption has been related to high density lipoprotein (HDL) cholesterol and its major subfractions, HDL_2 and HDL_3, measured in a single fasting blood sample.

The results confirm that high density lipoprotein cholesterol concentration increases as the amount of alcohol regularly consumed increases. The relationship appears to be linear and is independent of age, smoking habit, body mass index, low density lipoprotein cholesterol and plasma total triglyceride. This rise in HDL cholesterol is not mediated through either HDL_2 cholesterol or HDL_3 cholesterol alone. Both subclasses increase significantly and by similar amounts with increasing alcohol intake.

A number of cohort studies[1-3] have suggested that there is a U-shaped relationship between mortality from all causes and alcohol consumption. This U-shaped pattern arises from a combination of two opposing trends. Mortality from causes other than coronary heart disease rises with increasing alcohol consumption, the rise being particularly marked amongst the heaviest drinkers. Conversely, these studies and others[4] showed that incidence of coronary heart disease deaths and major non-fatal coronary events is highest amongst total abstainers, and decreases with increasing alcohol consumption.

Several cohort[5-7] and experimental[8-12] studies have shown that plasma high density lipoprotein (HDL) cholesterol concentration increases with increasing alcohol consumption. High levels of HDL cholesterol are associated with a lower incidence of coronary events,[13-17] although one recent study[16] has questioned whether this associatioin is independent of other factors. High density lipoprotein cholesterol could therefore be a link between higher alcohol consumption and lower coronary morbidity and mortality.[17] There is some evidence from case control studies[18 19] that the apparent protective effect of HDL cholesterol arises mainly in the HDL_2 subclass. The association between the HDL subclasses and alcohol is thus of particular interest.

None of the cohort studies[5 7] reported on the association between the HDL subclasses and alcohol consumption. Three[10-12] of the experimental studies did measure HDL subclasses, but with contradictory results. We describe in this report the relationships between the major HDL cholesterol subfractions and alcohol consumption in two large prospective cohort studies based in the town of Caerphilly in South Wales and in the Speedwell area of Bristol.

Subjects and methods

The populations and survey methods for the Caerphilly and Speedwell studies have been described

* Now at Department of Medicine, Bowman Gray School of Medicine, Wake Forest University, 300 South Hawthorne Road, Winston-Salem, North Carolina 27103.

in detail elsewhere.[20] Briefly, the population for the Caerphilly study was composed of men aged 45 to 59 years resident in the town of Caerphilly and five outlying villages. A total of 2512 men were seen—89% of the 2818 who were found to be eligible for inclusion. In Speedwell, a total sample of men from the practices of 16 general practitioners working from two Health Centres were chosen so that they were aged between 45 and 59 years on 1st September 1978, immediately before the study started. A total of 2348 men were seen—92% of the eligible population of 2550.

SURVEY METHODS

The men were invited to attend an afternoon or evening clinic for a general medical and cardiovascular examination. In Caerphilly, alcohol consumption was obtained as part of a self-administered diet questionnaire, which had been completed previously and which was checked at the clinic. In Speedwell the alcohol questionnaire was administered in the clinic by a nurse. Both questionnaires asked about the frequency of drinking alcohol, and the type and amount of alcohol usually drunk. From these questionnaires the volume of alcohol consumed was estimated as the number of millilitres per week.

A standard smoking questionnaire was also administered in both areas. Height, without shoes, was measured with a Holtain stadiometer, and weight was recorded using a beam balance.

The men were then asked to return, fasting, to an early-morning clinic in order to give a blood sample. Fasting samples were obtained from 2368 (94%) of the men in Caerphilly and from 2273 (97%) of the Speedwell men. Blood was taken with minimum venous stasis with the subject in the supine position. For the lipid measurements the blood was immediately anticoagulated with disodium ethylenediaminetetra-acetate (1 mg per ml). After centrifugation the plasma was transferred to plain plastic tubes, which were then despatched in crushed ice on the same day to the laboratories.

LABORATORY METHODS

All lipoprotein measurements were completed within 72 hours of venepuncture, plasma being maintained at 4°C while awaiting analysis.

Cholesterol and triglyceride concentrations were measured using enzymatic procedures (Boehringer-Mannheim; catalogue numbers 236691 and 644200 in Caerphilly; numbers 187313 and 166448 in Speedwell). The HDL fraction was isolated by precipitation of the very low and low density lipoprotein with sodium phosphotungstate and magnesium chloride[21] (Caerphilly) and with heparin and manganese chloride[22] (Speedwell). The HDL subclasses were isolated from the supernatant by ultracentrifugation in a Beckman Airfuge after adjusting the background density to 1·125 g/ml.[23] In Caerphilly the HDL_3 subclass (d 1·125–1·21 g/ml) was isolated, HDL and HDL_3 cholesterol were measured directly and the HDL_2 cholesterol was calculated by subtraction. In Speedwell the HDL_2 subclass (d 1·063– 1·125 g/ml) was isolated, HDL and HDL_2 were measured directly and HDL_3 cholesterol was calculated by difference.

STATISTICAL METHODS

The distributions of HDL cholesterol and its subfractions were all slightly skewed towards the higher values. The results presented here are from analyses based on natural units. The analyses were repeated using logarithmically transformed cholesterol concentrations, but this resulted in no material differences.

Both laboratories estimated one of the HDL cholesterol subfractions by subtracting the measured value of the other from the measured total HDL cholesterol. Because all measurements are subject to error, this subtraction procedure occasionally yielded a negative value. This was a very rare event for the Speedwell subjects, in whom the smaller HDL_2 subfraction was measured. Among the Caerphilly subjects, in whom the larger HDL_3 subfraction was assayed, it occurred in 4% of the men. Although clearly it is impossible for men to have negative values, they were included in the statistical analyses. The alternatives were to exclude the subjects with negative values or to re-code the negative values to zero, either of which would have distorted the distribution, and could have biased the association with alcohol consumption (though the relationship between HDL_2 cholesterol and alcohol in Caerphilly was not materially changed by recoding the negative values to zero).

Results

The distribution of alcohol consumption in the two populations is shown in table 1. Consumption was unknown for more men in Caerphilly than in Speedwell because of the method of collecting the data. The mean intake was 204 ml per week in Caerphilly, compared with 125 ml per week in Speedwell. Alcohol consumption decreased with age, and was associated with smoking habit. Ex-smokers, cigar and pipe smokers and light-to-moderate cigarette smokers tended to have similar intakes. Amongst those who had never smoked intake was about one-third lower, while amongst the heaviest cigarette smokers it was 40–50% higher.

J Epidemiol Community Health: first published as 10.1136/jech.42.3.220 on 1 September 1988. Downloaded from http://jech.bmj.com/ on May 29, 2023 by guest. Protected by copyright.

Table 1 *Distribution of alcohol consumption*

Alcohol (ml/week)	*Caerphilly* Number (%) of men	*Speedwell* Number (%) of men
0	148 (6·2%)	129 (5·5%)
1–9	166 (7·0%)	363 (15·5%)
10–24	279 (11·7%)	324 (13·8%)
25–49	212 (8·9%)	237 (10·1%)
50–99	262 (11·0%)	312 (13·3%)
100–149	261 (10·9%)	292 (12·4%)
150–199	250 (10·5%)	139 (5·9%)
200–299	297 (12·4%)	284 (12·1%)
300–499	268 (11·2%)	199 (8·5%)
500+	245 (10·3%)	68 (2·9%)
Total	2388	2347
Mean (SD)	204 (263)	125 (160)

Alcohol consumption was unknown for 124 (5%) men in Caerphilly and for 1 man in Speedwell.

Mean levels of HDL, HDL_2 and HDL_3 cholesterol are shown in table 2 for the two areas. Levels of HDL cholesterol were very similar in the two areas. Levels of HDL_2 cholesterol were 0·20 mmol/l higher in Caerphilly and levels of HDL_3 cholesterol were correspondingly 0·18 mmol/l lower. A comparability study in which 94 blood samples from Speedwell subjects were split and sent to both laboratories showed exactly similar differences: HDL_2 cholesterol was 0·17 mmol/l higher in the Caerphilly laboratory, HDL cholesterol was 0·01 mmol/l lower and HDL_3 cholesterol was 0·19 mmol/l lower. The conclusion from this is that the differences between the areas in the cholesterol concentration of HDL_2 and HDL_3 are not real but arise because of the differences in laboratory methods described above.

Table 2 *High density lipoprotein and subclass cholesterol concentrations (mmol/l)*

	Caerphilly Mean (SD)	*Speedwell* Mean (SD)
HDL cholesterol	1·12 (0·33)	1·11 (0·38)
HDL2 cholesterol	0·40 (0·30)	0·20 (0·14)
HDL3 cholesterol	0·73 (0·28)	0·91 (0·34)

Mean values of HDL cholesterol at ten different levels of alcohol consumption are shown in table 3, in which total abstainers have been separated from even the lightest of occasional drinkers. Mean HDL cholesterol increased steadily with increasing alcohol consumption, the heaviest drinkers having average values that were 0·2 to 0·3 mmol/l higher than those of the total abstainers and the very light drinkers. The similar data for HDL_2 and HDL_3 cholesterol are shown in fig 1. Here the mean cholesterol values have been transformed to standard deviation scores by subtracting the overall mean cholesterol value and then dividing by the overall standard deviation of the cholesterol. This eliminates the artificial differences in subclass cholesterol levels between the areas and permits direct comparison of the relationships. For both areas, and for both subfractions, the cholesterol levels increased steadily with increasing alcohol consumption.

Table 3 *High density lipoprotein cholesterol (mmol/l) and alcohol consumption*

Alcohol (ml/week)	*Caerphilly* N	*Caerphilly* HDL–C Mean (SD)	*Speedwell* N	*Speedwell* HDL–C Mean (SD)
0	138	1·07 (0·34)	120	1·03 (0·33)
1–9	155	1·03 (0·28)	341	1·05 (0·33)
10–24	261	1·02 (0·28)	307	1·07 (0·36)
25–49	200	1·05 (0·28)	228	1·11 (0·37)
50–99	247	1·11 (0·31)	295	1·09 (0·34)
100–149	247	1·13 (0·35)	277	1·11 (0·40)
150–199	232	1·15 (0·32)	133	1·14 (0·33)
200–299	277	1·16 (0·37)	265	1·13 (0·38)
300–499	249	1·17 (0·35)	182	1·25 (0·42)
500+	223	1·25 (0·36)	58	1·33 (0·57)
Total	2229	1·12 (0·33)	2206	1·11 (0·38)

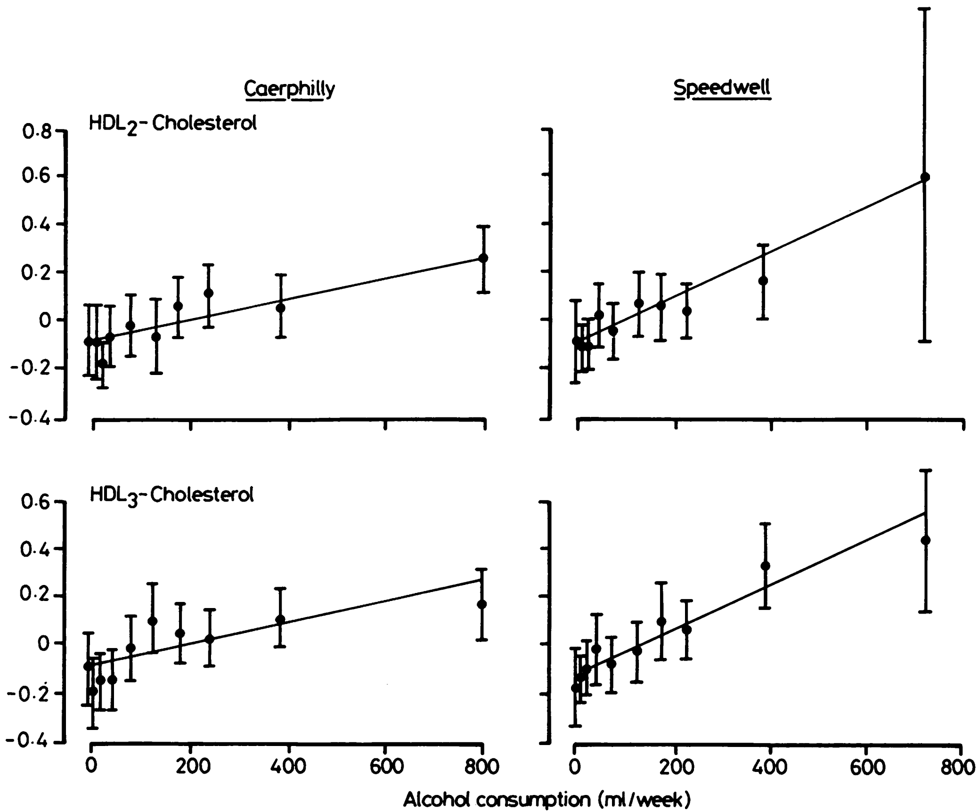
To quantify the associations between the cholesterol concentrations and alcohol better, a series of linear regression analyses was performed with cholesterol concentration as the dependent variable and alcohol consumption as the independent variable. The regression equations, in natural units, are given in table 4. For the two subclasses the regression equations are also shown in the figure, after transformation to standard deviation scores. The linear regression coefficients were, in all cases, highly statistically significant ($p < 0{\cdot}001$). The addition of quadratic terms to the model produced no improvement in the fit.

Table 4 *Associations between the high density lipoprotein cholesterols and alcohol consumption*

Linear regression equation	*t-value for regression coefficients**	*Difference in cholesterol level associated with 200ml difference in alcohol intake † (%)*
Caerphilly		
HDL–C = 1·069 + 0·00026 × Alcohol	8·7	16
HDL2–C = 0·368 + 0·00013 × Alcohol	4·9	9
HDL3–C = 0·705 + 0·00012 × Alcohol	4·7	9
Speedwell		
HDL–C = 1·060 + 0·00040 × Alcohol	7·5	21
HDL2–C = 0·188 + 0·00011 × Alcohol	5·6	16
HDL3–C = 0·877 + 0·00030 × Alcohol	5·9	18

* All regression coefficients are significantly different from zero at $p < 0{\cdot}001$
† Change in cholesterol level expressed as a percentage of the SD of the cholesterol distribution in the total population.

J Epidemiol Community Health: first published as 10.1136/jech.42.3.220 on 1 September 1988. Downloaded from http://jech.bmj.com/ on May 29, 2023 by guest. Protected by copyright.



High density lipoprotein subclass cholesterol concentrations and alcohol consumption. Mean value and 95% confidence interval within each of the 10 alcohol consumption groups as defined in table 3. (The cholesterol concentrations are given as standard deviation scores [see text]. The line shown is the linear regression of the cholesterol concentrations on alcohol consumption, based on individuals).

Because of the different levels of the various cholesterols it is inappropriate to use the regression coefficients in natural units to assess the relative magnitude of the association of the cholesterols with alcohol consumption. Therefore table 4 also shows the difference in cholesterol level associated with a 200 ml increase in alcohol consumption, this difference being expressed as a percentage of the standard deviation of the cholesterol distribution in the total population. As such, this difference is directly proportional to the regression coefficient when the cholesterol is expressed in standard deviation units, and is directly comparable for the different cholesterols. This index shows that the association between each of the cholesterols and alcohol was stronger in Speedwell than in Caerphilly. More importantly, it shows that HDL_2 and HDL_3 cholesterol had associations of very similar magnitude with alcohol consumption. Thus in Speedwell a difference of 200 ml/week in alcohol intake was associated with a difference in both HDL_2 and HDL_3 cholesterol of about 16–18% of one standard deviation. In Caerphilly the corresponding difference was 9% of one standard deviation for both subfractions.

Plasma total HDL cholesterol, HDL_2 cholesterol, HDL_3 cholesterol and alcohol consumption are all associated with other variables such as age, smoking habit, body mass index, low density lipoprotein cholesterol and plasma total triglyceride.[24] Triglyceride levels, which were negatively associated with HDL cholesterol and its subclasses, increased with increasing alcohol consumption. To assess the effect of these other variables on the associations between the HDL cholesterols and alcohol a series of multiple regression analyses was performed, in which each of the HDL cholesterols was taken as the dependent variable in turn.

In addition to showing that the association between the HDL cholesterols and alcohol was largely independent of their associations with other variables, the multiple regression analyses confirmed the well established[24] negative association between HDL

J Epidemiol Community Health: first published as 10.1136/jech.42.3.220 on 1 September 1988. Downloaded from http://jech.bmj.com/ on May 29, 2023 by guest. Protected by copyright.

cholesterol and body mass index. Body mass index was also negatively associated with both of the HDL subfractions. Smokers had levels of HDL and HDL_3 cholesterol that were about 0·04 to 0·07 mmol/l lower than non-smokers in both areas. In Speedwell, HDL_2 cholesterol was also lower by about 0·02 mmol/l among smokers. In Caerphilly there was no difference in the HDL_2 cholesterol levels of smokers and non-smokers.

Discussion

These studies confirm the well recognised association between HDL cholesterol concentration and alcohol consumption. In Caerphilly, HDL cholesterol increased by 0·026 mmol/l (1·0 mg/dl) with each increase of 100 ml/week in alcohol consumption. The corresponding figure in Speedwell was 0·040 mmol/l (1·5 mg/dl). These increases are at the lower end of the range of increases reported in the literature.[5–12] This mixture of small experimental studies and large observational cohort studies demonstrated increases in HDL cholesterol ranging from about 1·5 mg/dl to 3·0 mg/dl for an increase of 100 ml of alcohol per week. The present studies found no association between alcohol consumption and plasma total cholesterol concentration, and this is a consistent finding throughout the literature.[5 7–9 11–12] We found that total triglyceride increased with increasing alcohol consumption, as did both the other cohort studies[5 7] in which triglyceride was measured.

While the change in HDL cholesterol with alcohol consumption which we have observed is consistent with the existing literature, there was a substantial difference in the size of the effect between our two areas. This may have been a consequence of errors of measurement. The regression coefficients for alcohol should have been largely unaffected by random errors in the measurement of HDL cholesterol. However, they would have been affected by both random and systematic errors in the measurement of alcohol consumption. Random errors would have caused the regression coefficients to be under-estimated. The effect of any systematic errors is likely to have been greater, and dependent on their nature. It is generally assumed that alcohol consumption is likely to be under-reported. Systematic under-reporting will have caused the regression coefficients to be over-estimated. The mean alcohol consumption in Caerphilly was estimated as 204 ml per week, the corresponding figure in Speedwell being 125 ml. National expenditure figures for the United Kingdom[25,26] suggest that average consumption for men aged 45–64 years is likely to be at least 200 ml per week.

The measured alcohol consumption in Caerphilly was thus close to the estimated national average figure, while consumption among the Speedwell men was much lower. It is quite possible that this reflects the true state of affairs. However, it may be that the difference between the two areas arose because there was more under-reporting in Speedwell. The questionnaires used in the two areas were similar, but in Caerphilly it was self-administered and in Speedwell it was administered in the clinic by a nurse. This difference in method could have resulted in differential under-reporting. It is interesting to note that the standardised coefficients for the regression of HDL cholesterol on alcohol were 0·068 for the Caerphilly men and 0·064 for the Speedwell men. These coefficients are the estimated change in HDL cholesterol associated with a one standard deviation change in alcohol consumption. The overall conclusion must be that HDL cholesterol increases with increasing alcohol consumption, but that the exact size of the increase is rendered uncertain by imprecision and likely systematic biases in the measurement of alcohol consumption.

The main question that this study was designed to answer was whether the increase in HDL cholesterol associated with alcohol intake was mediated through the HDL_2 subclass or the HDL_3 subclass. In both our areas, both subfractions increased significantly and by similar amounts with increasing alcohol intake. Thus, in Caerphilly a difference of 200 ml in alcohol intake was associated with increases in both HDL_2 and HDL_3 cholesterol of nearly one tenth of a standard deviation. In Speedwell the corresponding increase in both subfractions was about one sixth of a standard deviation. As with HDL cholesterol it is possible that the apparently stronger effect of alcohol on HDL subclasses in Speedwell arose because of a greater degree of under-reporting of alcohol consumption.

There are few other studies with which we can compare our results. No other cohort study has examined the effect of alcohol on HDL subclasses. Two[10 11] of the three experimental studies which did measure HDL subclasses quantified total HDL_2 and HDL_3 mass by analytical ultracentrifugation, rather than HDL_2 and HDL_3 cholesterol. The one[12] experimental study which did measure HDL_2 and HDL_3 cholesterol suggested, tentatively, that an increase in HDL_2 cholesterol was the major component in the rise in HDL cholesterol induced by alcohol. The two large cohort studies described in the present report show that both HDL_2 cholesterol and HDL_3 cholesterol are increased with increasing alcohol consumption and that the relative increase is similar for the two subclasses. Further, we have shown that the associations of HDL cholesterol and its subclasses with alcohol consumption are independent

J Epidemiol Community Health: first published as 10.1136/jech.42.3.220 on 1 September 1988. Downloaded from http://jech.bmj.com/ on May 29, 2023 by guest. Protected by copyright.

of potentially confounding factors such as age, smoking habit, body mass index, low density lipoprotein cholesterol and plasma total triglyceride concentration.

In the Lipid Research Clinics follow-up study,[17] part of the effect of alcohol consumption on cardiovascular mortality was shown to be mediated through HDL cholesterol. A partial mediation of the effect is the most that could be expected if the protective effect of HDL cholesterol arose largely through the small HDL_2 subclass, as has been suggested.[18 19] However some studies[27] show both HDL_2 and HDL_3 cholesterol to be lower in subjects with evidence of ischaemic heart disease. Only one prospective study[28] has so far been reported. In this study total HDL subclass mass was measured rather than HDL subclass cholesterol and it showed that both HDL_2 and HDL_3 mass were reduced in men who later developed ischaemic heart disease. The first follow-up phase of the two prospective studies reported here is nearly complete and will provide much needed information on the inter-relationships between the HDL cholesterol subclasses, alcohol intake and ischaemic heart disease.

Correspondence to: Peter M. Sweetnam, MRC Epidemiology Unit, 4 Richmond Road, Cardiff CF2 3AS.

References

1 Blackwelder WC, Yano K, Rhoads GG, Kagan A, Gordon T, Palesch Y. Alcohol and mortality: the Honolulu heart study. *Am J Med* 1980; **68:** 164–9.

2 Klatsky AL, Friedman GD, Siegelaub AB. Alcohol and mortality. A ten-year Kaiser-Permanente experience. *Ann Intern med* 1981; **95:** 139–45.

3 Marmot MG, Rose G, Shipley MJ, Thomas BJ. Alcohol and mortality: A U-shaped curve. *Lancet* 1981; i: 580–3.

4 Kozarevic D, Demirovic J, Gordon T, Kaelber CT, McGee D, Zukel WJ. Drinking habits and coronary heart disease. The Yugoslavia cardiovascular disease study. *Am J Epidemiol* 1982; **116:** 748–58.

5 Castelli WP, Doyle JT, Gordon T, *et al.* Alcohol and blood lipids. The cooperative lipoprotein phenotyping study. *Lancet* 1977; ii: 153–5.

6 Gordon T, Ernst N, Fisher M, Rifkind BM. Alcohol and high density lipoprotein cholesterol. *Circulation* 1981; **64 (Suppl III):** 63–6.

7 Thelle DS, Shaper AG, Whitehead TP, Bullock DG, Ashby D, Patel I. Blood lipids in middle-aged British men. *Br Heart J* 1983; **49:** 205–13.

8 Thornton J, Symes C, Heaton K. Moderate alcohol intake reduces bile cholesterol saturation and raises HDL cholesterol. *Lancet* 1983; ii: 819–21.

9 Belfrage P, Berg B, Hagerstrand I, Nilsson-Ehle P, Tornqvist H, Wiebe T. Alterations of lipid metabolism in healthy volunteers during long-term ethanol intake. *Eur J Clin Invest* 1977; **7:** 127–31.

10 Fraser GE, Anderson JT, Foster N, Goldberg R, Jacobs D, Blackburn H. The effect of alcohol on serum high density lipoprotein (HDL): A controlled experiment. *Atherosclerosis* 1983; **46:** 275–86.

11 Haskell WL, Canargo C, Williams PT, Vranizan KM, Krauss RM, Lindgren FT, Wood PD. The effect of cessation and resumption of moderate alcohol intake on serum high-density-lipoprotein subfractions. *N Engl J Med* 1984; **310:** 805–10.

12 Burr ML, Fehily AM, Butland BK, Bolton CH, Eastham RD. Alcohol and high-density-lipoprotein cholesterol: a randomized controlled trial. *Br J Nutr* 1986; **56:** 81–6.

13 Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. *Am J Med* 1977; **62:** 707–14.

14 Yaari S, Goldbourt U, Even-Zohar S, Neufeld HN. Associations of serum high density lipoprotein and total cholesterol with total, cardiovascular, and cancer mortality in a 7-year prospective study of 10,000 men. *Lancet* 1981; i: 1011–4.

15 Miller NE, Førde OH, Thelle DS, Mjøs OD. The Tromsø heart study. High-density lipoprotein and coronary heart disease: a prospective case-control study. *Lancet* 1977; i: 965–7.

16 Pocock SJ, Shaper AG, Phillips AN, Walker M, Whitehead TP. High density lipoprotein cholesterol is not a major risk factor for ischaemic heart disease in British men. *Br Med J* 1986; **292:** 515–9.

17 Criqui MH, Cowan LD, Tyroler HA, Bangdiwala S, Heiss G, Wallace RB, Cohn R. Liproproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results from the Lipid Research Clinics Follow-up study. *Am J Epidemiol* 1987; **126:** 629–37.

18 Ballantyne FC, Clark RS, Simpson HS, Ballantyne D. High density and low density lipoprotein subfractions in survivors of myocardial infarction and in control subjects. *Metabolism* 1982; **31:** 433–7.

19 Miller NE, Hammett F, Saltissi S, Rao S, Van Zeller H, Coltart J, Lewis B. Relation of angiographically defined coronary artery disease to plasma lipoprotein subfractions and apolipoproteins. *Br Med J* 1981; **282:** 1741–4.

20 Yarnell JWG, Elwood PC, Sweetnam PM, *et al.* Caerphilly and Speedwell collaborative heart disease studies. *J Epidemiol Community Health* 1984; **38:** 259–62.

21 Lopes-Virella M F, Stone P, Ellis S, Colwell J A. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin Chem* 1977; **23:** 882–4.

22 Warnick GR, Albers JJ. A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J Lipid Res* 1978; **19:** 65–76.

23 Eyre J, Hammett F, Miller NE. A micro-method for the rapid ultra-centrifugal separation of human plasma high density lipoprotein subfractions, HDL_2 and HDL_3. *Clin Chim Acta* 1981; **114:** 225–31.

24 Miller NE, Miller GJ, Eds. *Clinical and metabolic aspects of high density lipoproteins.* Amsterdam: Elsevier, 1984.

25 Kaelber C, Mills G. Alcohol consumption and cardiovascular diseases: Introductory remarks. *Circulation* 1981; **64: (Suppl. III):** 1–6.

26 Central Statistical Office. *Social trends No 15, 1985 Edition.* London: HMSO, 1985.

27 Wallenten L, Sundin B. HDL_2 and HDL_3 lipid levels in coronary artery disease. *Atherosclerosis* 1985; **59:** 131–6.

28 Gofman JW, Young W, Tandy R. Ischaemic heart disease, atherosclerosis and longevity. *Circulation* 1966; **34:** 679–97.

Accepted for publication April 1988

J Epidemiol Community Health: first published as 10.1136/jech.42.3.220 on 1 September 1988. Downloaded from http://jech.bmj.com/ on May 29, 2023 by guest. Protected by copyright.