SERUM CHOLESTEROL, SMOKING, AND BODY BUILD A SURVEY IN THE ROYAL AIR FORCE

BY

J. C. McDONALD*

Epidemiological Research Laboratory, Colindale

O. M. LIDWELL

Cross-Infection Reference Laboratory, Colindale

AND

E. A. WRIGHT

St. Mary's Hospital, London

This study was made to elucidate the relationship between serum cholesterol concentration and other factors such as smoking and body build which are also associated with coronary artery disease. Where the variable of interest, in this case the serum cholesterol concentration, is or may be dependent on a number of factors which are themselves correlated, it is essential to analyse the data in a way which permits an estimate to be made of the effect of each factor separately. The method used to achieve this is described below.

In the course of a survey of respiratory virus infection in the Royal Air Force (McDonald, Miller, Zuckerman, and Pereira, 1962), blood specimens were taken from almost a thousand healthy men. The opportunity was taken to ask the men a few additional questions at the time they were bled and some serum was put aside for cholesterol estimation.

MATERIALS AND METHODS

SPECIMENS.—In January, 1960, volunteers were sought among 500 new recruits to the RAF and 222 agreed to be bled. All but two of the men were under 30 years of age and at the time had been in the Service one week. In the same month a further 764 volunteers of varied age and rank were found and bled in ten operational stations. The recruits were drawn from all parts of the British Isles and the operational stations were widely scattered geographically, but there can be no assurance that the volunteers were representative of the populations from which they were drawn. The blood specimens were

taken in the early morning or in the early afternoon and virtually all the volunteers would have had either breakfast or their mid-day meal 1 or 2 hours earlier; the blood was taken into vacuum tubes which were kept at the ambient temperature in transit to the laboratory where they arrived up to 24 hrs later. After separation, serum was stored at -20°F . until tested in the laboratory in the autumn of 1961. Of the 986 men who were bled, specimens from 976 were available for examination.

RECORDS.—Immediately before the blood was taken a note was made of the volunteer's rank and he was asked to give his age, height, weight, and smoking habits. The recruits were also asked the age at which they left school and their occupation immediately before joining the Service. A few volunteers who seemed uncertain about their height or weight were measured—height in bare feet and weight in pants only. As servicemen are weighed and measured frequently the volunteers' statements were probably reasonably accurate.

CHOLESTEROL ESTIMATION.—Immediately before being tested the specimens were thawed in water at room temperature. The cholesterol concentration was estimated by the method of Zlatkis, Zak, and Boyle (1953) as modified by Henly (1957), using an Eel colorimeter and an Ilford No. 626 filter. This method measures total cholesterol and cholesterol esters and, although not entirely specific, is not influenced by lipaemia or severe icterus (Chiamori and Henry, 1959). These authors state that a 10 per cent. positive error can be caused by 1 per cent. haemoglobin, by

* Present Address: Department of Epidemiology and Health, McGill University, Montreal.

10 mg. bromide as Na Br per 100 ml., or by 10 mg. iodide per 100 ml. Our most severely haemolysed specimen contained less than 0.5 per cent. haemoglobin and of fourteen other specimens showing lesser degrees of haemolysis only two contained more than 0.1 per cent. haemoglobin. Significant levels of iodide are not encountered in sera and the unlikely presence of bromide would have been detected by a change of the colour of the supernatant (Chiamori and Henry, 1959). Three standard concentrations of cholesterol were included in each day's batch of tests and these were found not to vary during the investigation which was completed in 21 working days of one month. Approximately fifty specimens were tested each day; these usually comprised 45 new specimens and at least three sera which had been tested the day before. Sera for the repeat tests were selected at random from bottles containing plenty of serum and tended to be taken from specimens tested during the second half of the day before. This was not considered to have introduced any bias. In all, 78 (8 per cent.) of the specimens were tested twice; the standard error of a single estimation, deduced from the replicate readings, was 3.2 per cent.

STATISTICAL ANALYSIS.—As an indication of body build, the ponderal index (Sheldon, Stevens, and Tucker, 1940) was calculated for each man by dividing height in inches by the cube root of weight in pounds. Those who were heavy for their weight had therefore a low ponderal index and *vice versa*. The men were classified as ex-smokers (59 men), non-smokers (234 men), and smokers (683 men). The smokers were further divided into three grades—light, moderate, and heavy—according to the number of cigarettes smoked per day. Light smokers were those smoking less than 15 cigarettes a day, moderate 15 to 24, and heavy 25 or more. Fifteen cigarette-smokers also smoked a pipe; one ounce (28 g.) of tobacco was considered to be equivalent to 28 cigarettes. The civilian occupations of the recruits were divided according to the physical activity involved into three groups—light, medium, and heavy. Using school-leaving age as a rough socio-economic index, the recruits were further divided into five groups—under 15, 15, 16, 17, and 18 years or more.

For the 976 men from whom serum had been tested information on nine characteristics was available for analysis. Seven of these—age, weight, ponderal index, amount smoked, school-leaving age, occupational activity, and serum cholesterol—were or could be treated as continuous variables, and two—rank and the status of ex-smoker—had to be considered in a number of discrete classes. Regression

equations for the relationship between the cholesterol values and all the variables were obtained with the help of an electronic computer. As a first approximation, the values of the cholesterol concentration were expressed in the form of a linear regression equation of the form:

$$y = a + b_1x_1 + b_2x_2 + b_3x_3 + \dots$$

where y is the estimated value of the variable when x_1, x_2, x_3 , etc., are the values of the factors 1, 2, 3, etc., and a, b_1, b_2, b_3 , etc., are constants.

Convenient methods for the evaluation of these constants, where some of the factors are continuous variables and others represent distinct states, are discussed by Lidwell (1961). When there are more than two or three factors involved, the necessary computations become very tedious, or quite impracticable, unless they can be performed by electronic methods.

RESULTS

The data from the eight sub-groups, comprising the four ranks, officers, NCOs, recruits, and other ranks, each divided into ex-smokers and smokers or non-smokers, were subjected separately to a similar analysis. There were only 59 ex-smokers altogether and no significant regression coefficients were found in these four sub-groups. Each of the four groups of smokers or non-smokers showed significant regressions with age and ponderal index but with no other factors. In those recruits who were smokers or non-smokers (there were only seven ex-smokers), the regression coefficient with school-leaving age was 1.43 (standard error 2.4) and with pre-enlistment activity, 1.22 (standard error 3.6). As these factors were not significantly related to cholesterol values they were omitted from subsequent analyses. Since the analyses of the data from the four ranks separately did not show any significant differences in the relationship between the various factors examined and the blood cholesterol levels for the different ranks, the data were combined. For the group of ex-smokers as a whole, the only significant regression coefficient was that for age; for smokers and non-smokers there were significant correlations with age and ponderal index and there was also a significant difference between recruits and other ranks. There was however no evidence of any significant difference between ex-smokers and the rest in the relationship between the various factors and the blood cholesterol levels, so a final regression analysis was carried out on the combined data from the whole series of 976 men.

The findings from this analysis are summarized in Table I. No significant regression was found with smoking in the whole group; none had been found previously in any of the groups classified by rank. The regression on weight was small and of doubtful significance. Recruits as a class had significantly lower cholesterol levels than trained personnel of whatever rank, but there was no significant difference between officers, NCOs, and other ranks (excluding recruits).

TABLE I
RELATION OF SERUM CHOLESTEROL VALUES TO OTHER FACTORS

This is expressed in the form of a regression equation whereby serum cholesterol in mg. per 100 ml. = $a + b_1x_1 + b_2x_2 + b_3x_3 + \dots$

Variables (x_1 to x_4)*	Mean Values	Regression Coefficient†	Standard Error
Age (yrs) ($\bar{x}_1 + x_1$)	23.8 ($\bar{x}_1$)	2.64 (b_1)	0.23
Weight (lb.) ($\bar{x}_2 + x_2$)	157 ($\bar{x}_2$)	0.10 (b_2)	0.07
Ponderal Index ($\bar{x}_3 + x_3$)	13.1 ($\bar{x}_3$)	-17.7 (b_3)	2.9
Amount Smoked ($\bar{x}_4 + x_4$)	1.1 ($\bar{x}_4$)	1.73 (b_4)	1.5
Ex-Smoker (x_5)	—	1.12 (b_5)	5.3
Rank { Officer (x_6)	—	4.7 (b_6)	2.6
NCO (x_7)	—	-1.3 (b_7)	2.7
Recruit (x_8)	—	-9.6 (b_8)	2.8
Other (x_9)	—	4.1 (b_9)	2.5
Mean Cholesterol Level (mg./100 ml.) (smokers and non-smokers) ..	215.1 (a)		
Mean Square (about mean) ..	1,916		
Residual Variance ..	1,369		

* x_1 to x_4 are expressed as differences from the mean values.
 x_5 takes the value of 0 for non-smokers, 1 for light smokers, 2 for moderate smokers, and 3 for heavy smokers.
 x_6 takes the value of 1 for ex-smokers and is zero for all others.
 x_7 takes the value of 1 for officers and is zero for all others.
 x_8 takes the value of 1 for NCOs and is zero for all others.
 x_9 takes the value of 1 for recruits and is zero for all others.
 x_{10} takes the value of 1 for other ranks and is zero for all others.

† Coefficients which exceed twice their standard error and may therefore be regarded as significant are in *italics*.

The regressions on age and ponderal index, however, were clear and consistent. Rather less than 30 per cent. of the variance about the mean was absorbed by the factors included in the analysis, and this was

almost entirely (96 per cent.) due to age and ponderal index. The residual variance (1,369) corresponds to a coefficient of variation of 17 per cent. which is substantially in excess of the error of replicate determinations (3.2 per cent.). The factors responsible for the residual variation have yet to be defined; some at least must be attributable to genetic factors.

The distribution of mean cholesterol values in relation to the two dominant variables, age and ponderal index, is shown in Table II.

DISCUSSION

The results of other surveys in relation to body build and smoking have been conflicting, but on these issues our findings seem quite clear. This may have been due in part to the employment of multiple regression analysis; the very poor correlation with weight contrasted with the close relationship to ponderal index. Though most investigators, see Kontinen and Rajasalmi (1963) and Report of the Advisory Committee to the Surgeon General of the USPHS (1964), have reported that smokers have higher cholesterol levels, our entirely negative findings are in line with those of Kontinen (1962), who studied young servicemen in Finland, and Acheson and Jessop (1961) who examined a group of Dublin pensioners. If there is indeed no constant correlation between serum cholesterol values and smoking, the association that has been observed between coronary artery disease and these factors (Hammond and Horn, 1958; Doyle, Dawber, Kannel, Heslin, and Kahn, 1962; Doll and Hill, 1964) must depend on separate and independent relationships.

Our results in relation to age are similar to those from most large surveys of urban males in countries such as New Zealand (Hunter and Wong, 1961) and the United States (Schilling, Christakes, Bennett, and Coyle, 1964). We could not confirm the finding of these and other workers of a plateau or fall in

TABLE II
MEAN SERUM CHOLESTEROL RELATED TO AGE AND PONDERAL INDEX

Age (yrs)	Ponderal Index*						All Men	
	Less Than 12		12		13+			
	Tested	Mean (mg. %)	Tested	Mean (mg. %)	Tested	Mean (mg. %)	Tested	Mean (mg. %)
17-19	2	215	87	203	124	187	213	194
20-29	21	248	307	220	296	204	624	213
30-39	14	283	58	254	28	239	100	254
40-52	5	282	28	267	6	265	39	269†
All Ages	42	262	480	224	454	203	976	216

* height (in.).

† $\sqrt{\text{weight (lb.)}}$

† 267 mg. per cent. for 23 men aged 40-44 and 273 mg. per cent. for 16 men aged 45-52.

mean cholesterol values in older men. The oldest subject in our study was, however, only 52 years of age and there was some evidence of a decline in the rate of increase in men of 40 and over.

SUMMARY

Serum cholesterol concentration was estimated in 976 RAF men aged 17 to 52 years. Multiple regression analysis showed a consistent and highly significant correlation between mean serum cholesterol and age and ponderal index ($\frac{\text{height}}{\sqrt{\text{weight}}}$), but little relationship to weight and none to smoking habits or—in recruits—to school-leaving age or pre-enlistment occupation. Recruits had significantly lower cholesterol levels than officers, NCOs, or other ranks independently of any differences in age or ponderal index.

We are indebted to officers and men of the Royal Air Force for enabling us to make this study; to Dr V. H. T. James, St Mary's Hospital, London, for advice, to Dr D. L. Miller who helped to collect the specimens, to Miss Edith Trip for testing them so efficiently, and to Mrs Judith Munk for much computational assistance. We are also grateful to the St Mary's Hospital Endowment Fund for financial support and to Mr J. H. A. Dunwoody and the Rothamsted Experimental Station for the work done with the electronic computer. We thank the Director

General of the Royal Air Force Medical Services for permission to publish this report.

REFERENCES

- Acheson, R. M., and Jessop, W. J. E. (1961). *Brit. med. J.*, **2**, 1108.
 Chiamori, N., and Henry, R. J. (1959). *Amer. J. clin. Path.*, **31**, 305.
 Doll, R., and Bradford Hill, A. (1964). *Brit. med. J.*, **1**, 1399 and 1460.
 Doyle, J. T., Dawber, T. R., Kannel, W. B., Heslin, A. S., and Kahn, H. A. (1962). *New Engl. J. Med.*, **266**, 796.
 Hammond, E. C., and Horn, D. (1958). *J. Amer. med. Ass.*, **166**, 1159.
 Henly, A. A. (1957). *Analyst*, **82**, 286.
 Hunter, J. D., and Wong, L. C. K. (1961). *Brit. med. J.*, **2**, 486.
 Kontinen, A. (1962). *Ibid.*, **1**, 1115.
 — and Rajasalmi, M. (1963). *Ibid.*, **1**, 850.
 Lidwell, O. M. (1961). *J. Hyg. (Lond.)*, **59**, 259.
 McDonald, J. C., Miller, D. L., Zuckerman, A. J., and Pereira, M. S. (1962). *Ibid.*, **60**, 235.
 Report of the Advisory Committee to the Surgeon General of the U.S. Public Health Service (1964). "Smoking and Health". Public Health Service Publication, No. 1103. U.S. Government Printing Office, Washington.
 Schilling, F. J., Christakis, G. J., Bennett, N. J., and Coyle, J. F. (1964). *Amer. J. publ. Hlth*, **54**, 461.
 Sheldon, W. H., Stevens, S. S., and Tucker, W. B. (1940). "The Varieties of Human Physique", p. 266. Harper, New York and London.
 Zlatkis, A., Zak, B., and Boyle, A. J. (1953). *J. Lab. clin. Med.*, **41**, 486.