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EFFECT OF APPLES ON SERUM CHOLESTEROL LEVELS IN HUMANS

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ABSTRACT

Seventy-six free living male volunteers in the age group 30 to 50 years were paired in two groups based on similar cholesterol levels. Each individual in one group ate at least two apples per day over the period November 17 to March 10 while the corresponding individuals in the other group ate only three apples, or their fruit equivalent, per week. No other dietary control was exerted on the subjects.

Serum cholesterol levels were measured every 3 to 4 weeks during the experiment and the apple group had consistently lower mean levels, significantly so on the last two analysis dates, than the 'non' apple group. The largest difference found in mean serum cholesterol levels between the groups was 8.1%.

The results of this experiment could have application in the context of long-term modification of dietary patterns in relation to heart disease risk both in a general community sense and also in hyperlipidaemic subjects.

INTRODUCTION

There have been a number of reports recently concerning the reduction of serum cholesterol levels in humans who have been fed on diets high in dietary fibre, specifically bran (1), pectin (2, 3, 4, 5, 6, 7) and soya protein (8). In most of these experiments the dietary element under study has been extracted and administered under artificially controlled conditions. While this approach has tested the therapeutic effects of the substance in isolation it does not test the feasibility of its administration or the effectiveness of its use as part of the normal diet in a free living population.

It was decided, therefore, to test the effectiveness of a food containing pectin i.e. apples, as a cholesterol-lowering agent when incorporated in a normal diet. From October 1976 to March 1977, a semi-controlled study was carried out with matched pairs of volunteers to test the cholesterol-lowering effects of diets low and high in apple content under normal living conditions. Apples have the advantage of being highly acceptable, freely available during most of the year, and convenient to consume. Also Italian workers in 1962 showed that pulped apples consumed after each meal reduced serum cholesterol levels (9).

EXPERIMENTAL

Volunteers for study

Male volunteers in the age group 30 to 50 years in three Research Centres and the Administrative Centre of the Agricultural Institute in Dublin participated in the study. Their weight, height, smoking habits and approximate intake of dietary fibre were measured at the start of the experiment and their weight and intake of non-fruit dietary fibre was recorded again at the end of the experiment. Approximate dietary fibre intake of the subjects was obtained by basing it on their weekly intake of fruit, vegetables, cereals and cereal products. A weight-height index $(W/H)^2$ as used by Goldbourt and Medalie (10) was used in this test as an index of general nutritional status and build. W is expressed in grams and H in cm.

Experimental design

Eighty male volunteers were obtained for the study giving a total of 40 pairs. Two volunteers dropped out during the experiment, reducing the number of groups to 38. All the 76 volunteers were in the age group 30 to 50 years with the exception of 7; 6 of these were less than 30 and 1 was over 50 years. Twelve of the volunteers smoked cigarettes, only 2 of them in excess of 20 per day, 5 were pipe smokers and 5 smoked cigars. The confounding effect of smoking was thus minimal in this study.

Two preliminary serum cholesterol analyses were carried out a week apart at the end of October, 1976 and the mean values were calculated. Volunteers were then paired based on similar serum cholesterol levels. A second factor used for pairing was similar fruit dietary fibre intake. The decision as to which person of each pair would be on the apple diet was decided in a random fashion except in five instances where one person in each pair requested to be on the apple diet, or not to be on the apple diet, for personal reasons.

Apple intake

The apple intake of each individual in the apple group, termed (+) apple group in this paper, was based on fruit intake before the experiment began. Each volunteer in the apple group was requested to eat two apples per day in addition to what he was consuming before the experiment started. The total daily intake of this group thus varied from a minimum of two per day and permitted limited observation of a dose response relationship.

The apples, Irish Golden Delicious (diameter 70 to 75 mm) were supplied on a weekly basis, i.e. 14 per week to each individual, together with a log sheet to record the daily total fresh fruit intake. No further dietary control was exerted and the apples were continued daily from November 17, 1976, to March 10, 1977.

The people in the non-apple group, termed (–) apple group in this paper, were requested not to eat more than three apples or their fruit equivalent per week. They were also issued with a log sheet to record daily fruit intake.

Cholesterol and high density lipoprotein cholesterol (HDLP cholesterol) analyses

In addition to the preliminary samples taken in October blood samples were taken on December 9, January 13, February 17, March 10 and three weeks after cessation of the apple diet on March 31 and serum cholesterol was measured on each of these occasions. HDLP cholesterol was measured on March 10 and March 31 only.

Blood samples were allowed to clot and the serum was separated after 4 hr. Serum was stored at 4°C and analysed within 24 hr. Serum cholesterol measurements were performed in duplicate by the Unicam method (11) and the results expressed in mg per 100 ml. Serum HDLP cholesterol was measured by the method of Burnstein *et al* (12) and results were expressed in mg per 100 ml. These data were combined for the purpose of this paper and HDLP cholesterol is expressed as a percentage of total cholesterol.

The analyses were blind in that each sample was allotted a different number on each occasion and laboratory identification was impossible until the survey was completed.

Because of the possible link (13) between the % HDLP cholesterol level and level of physical activity the (+) and (–) apple groups were asked to fill in a questionnaire as to their approximate weekly level of exercise in the month of March 1977.

Analysis of results

The individual serum cholesterol levels of volunteers in the (+) and (–) apple groups were compared using a paired *t* test at each analysis date.

Correlation co-efficients were calculated for the following: (W/H)² values x initial cholesterol values; apple consumption of individuals in the period prior to each cholesterol analysis x the individual cholesterol figures for the period; (W/H)² for the (+) and (–) apple groups x the corresponding cholesterol levels for the March 10 analysis date.

RESULTS

Dietary fibre – apple intake

The approximate weekly-non-fruit dietary fibre intake of the (+) and (–) apple groups was similar at the start of the experiment and also at the end. The mean daily apple intake target for persons in the (+) apple group was three apples per day based on apple consumption before the experiment i.e. one per day on average plus two additional ones

for the experiment. In contrast the apple intake target, or fruit equivalent, of individuals in the (-) apple group was not more than three apples per week. The actual apple intake of the two groups during the course of the experiment is shown in Table 1. The results show that at least 80% of the required number of apples was consumed by the (+) apple group in each of the four periods prior to the cholesterol analyses (Table 1). Individuals in the (-) apple group only exceeded their allowed intake of three apples or fruit equivalent per week by a small amount (Table 1).

TABLE 1: Percentage of target^a apple consumption achieved by the two groups in the periods prior to each cholesterol analysis date

	Period			
	1	2	3	4
	Nov. 17-Dec. 8	Dec. 9-Jan. 12	Jan. 13-Feb. 16	Feb. 17-March 10
(+) apple group	90	80	83	86
(-) apple group	107	100	106	106

^aAverage target of (+) apple group, 20 apples/person/week
 " " "(-) " " , not more than 3/person/week.

Apples and serum cholesterol levels

The data for the serum cholesterol levels of the (+) and (-) apple groups during the course of the experiment are shown in Table 2. The values are expressed in graphic form in Fig. 1, which shows that the serum cholesterol values for the (+) apple group were consistently lower than those for the (-) apple group during the course of apple intake. The difference was significant on February 17 and March 10 analyses dates. Three weeks after the apple diet was finished the (+) apple group had a higher mean serum cholesterol level than the (-) apple group but not significantly so. The actual data for each individual are shown in scatter diagrams (Figs. 2a-f) where the serum cholesterol data for the (+) apple group are plotted against those for the (-) apple group for each analysis date.

TABLE 2: Serum cholesterol levels (mg/100 ml) in the apple experiment

Date	(+) apple group	(-) apple group	Paired <i>t</i> test	SE (±)
Prelim. October	205	204	NS	0.89
Dec. 9	223	234	NS	4.02
Jan. 13	234	235	NS	4.41
Feb. 17	231	242	*	3.13
Mar. 10	210	227	**	3.63
Mar. 31 ^a	230	224	NS	3.65

^aThree weeks after cessation of the apple diet

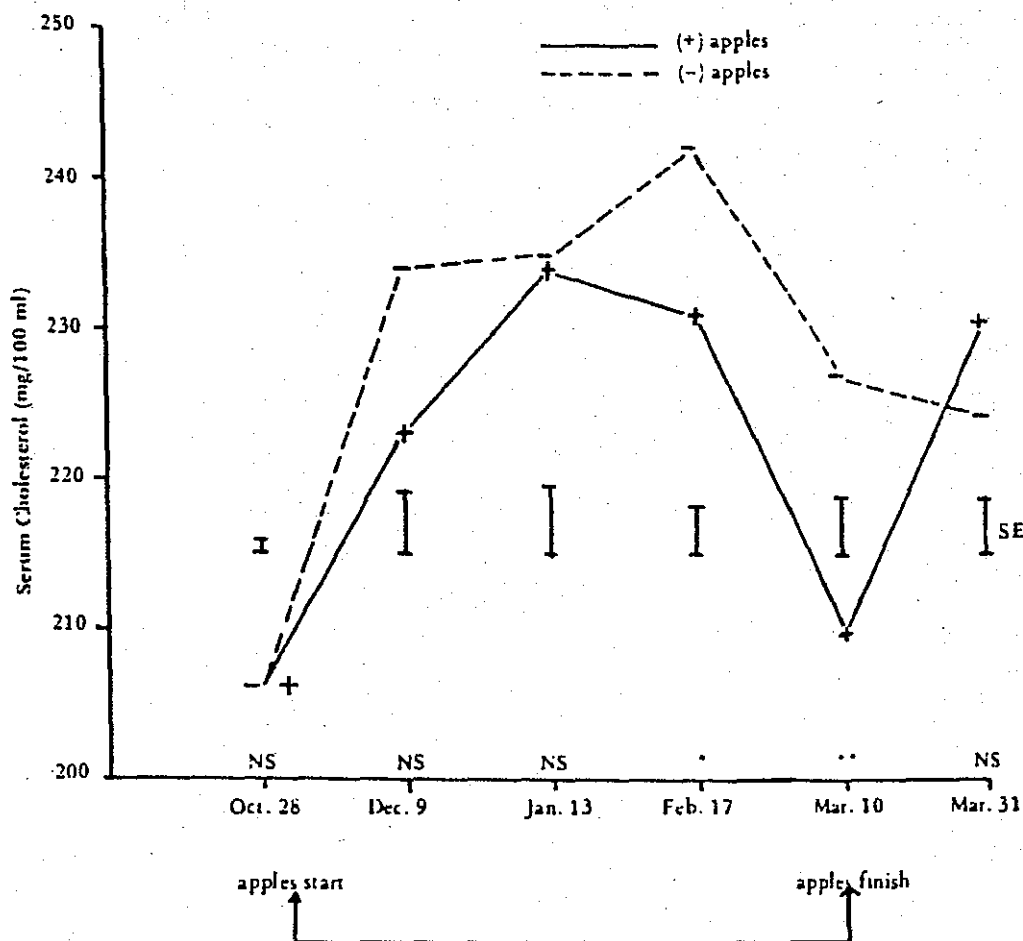


Fig. 1: Apple diet and serum cholesterol levels

Fig. 2a shows the average of two preliminary analyses. Points which fall on the diagonal lines indicate a pair with identical serum cholesterol levels; points above the line indicate pairs in which the person on the apple diet had a lower serum cholesterol level; points below the line indicate pairs in which the person on the non-apple diet had the lower serum cholesterol level. Figs 2a-f show the following: Fig. 2a—indicates the closeness of the initial pairing; Fig. 2b—a non-significant trend in which people on apples tended to have lower serum cholesterol levels than those on the non-apple diet; Fig. 2c—no trend visible; Fig. 2d—as in Fig. 2b except trend is significant; Fig. 2e—as in Fig. 2d; and Fig. 2f shows a non-significant trend in which people who had been on the apple diet tended to have a higher serum cholesterol level than those who were on the non-apple diet. The data also indicate that the effect due to apples occurred at the low end of the serum cholesterol range in addition to the high end; in pairs with low serum cholesterol levels the person on apples was still likely to have a lower value than the person on the low apple intake.

Apples, exercise and % HDLP cholesterol

The (+) apple group had a significantly higher % HDLP cholesterol level than the (-)

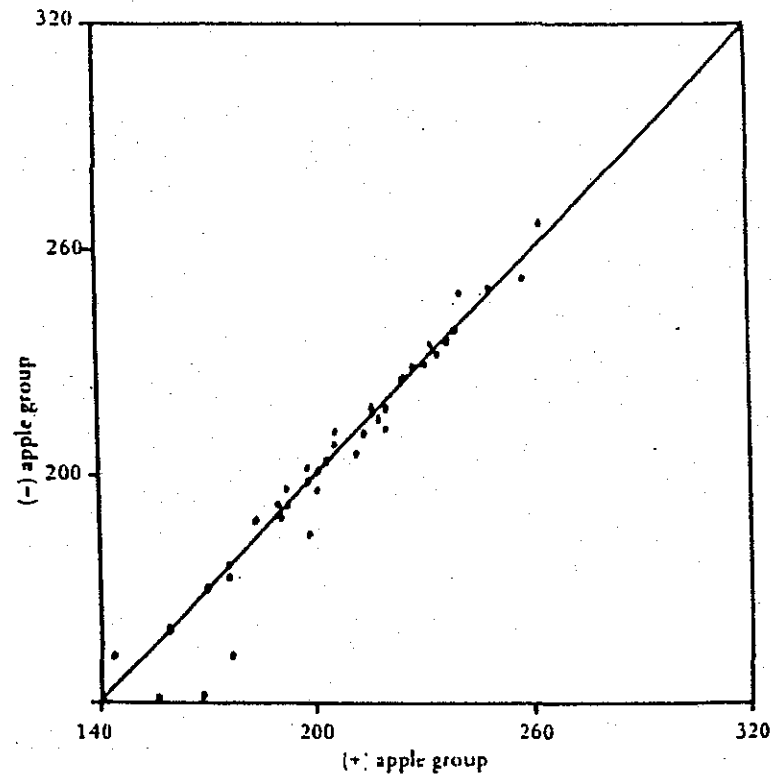


Fig. 2a: Scatter diagram for individual serum cholesterol levels (mg/100 ml) October 28

apple group (Table 3) at the end of the apple diet (March 10). Three weeks later there was still no difference. There was no clearcut relationship between the approximate exercise levels and the corresponding HDLP cholesterol values. The % HDLP cholesterol levels are presented in scatter diagram form in Figs. 3a-b.

Weight

There was no statistically significant difference between $(W/H)^2$ indices of the (+) and (-) apple groups, at 2.46, and 2.49 respectively at the start of the experiment, or at the end, at 2.52 and 2.53 respectively. The mean values for both groups increased during the experiment.

Correlation co-efficients

Correlation co-efficients calculated for a number of factors outlined in the Experimental section were all non-significant. The only point of note was that the correlation co-

TABLE 3: Percentage high density lipoprotein cholesterol levels in the apple experiment

Date	(+) apple group	(-) apple group	Paired <i>t</i> test	SE (\pm)
Mar. 10	24	21	*	0.89
Mar. 31 ^a	18	18	NS	0.75

^aThree weeks after cessation of the apple diet

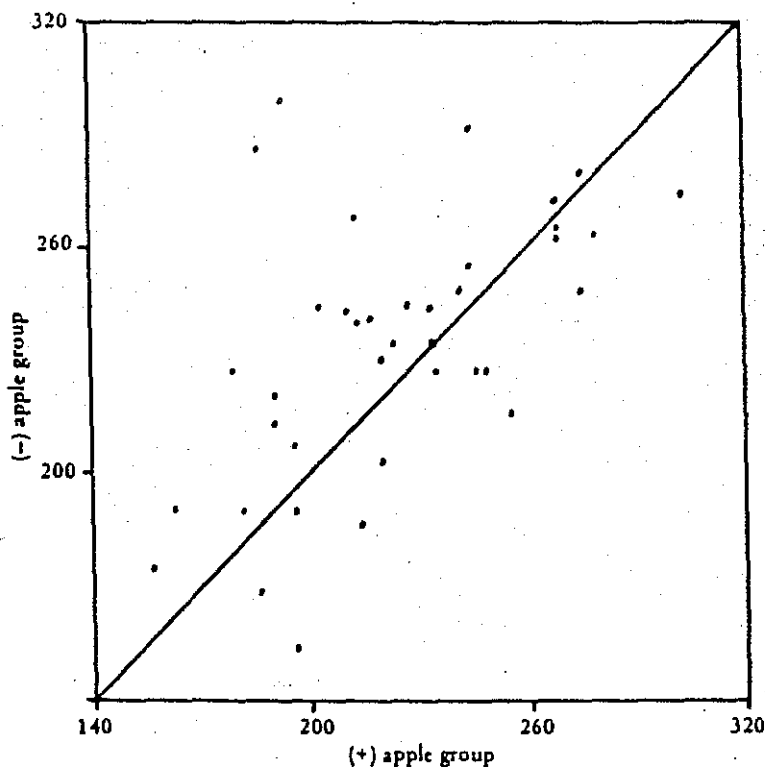


Fig. 2b: Scatter diagram for individual serum cholesterol levels (mg/100 ml) December 9

efficients between individual serum cholesterol levels and fruit consumption in the period prior to each analysis date were all negative in the case of the (+) apple group and positive for the (-) apple group.

DISCUSSION

The results of this experiment suggest that two to three apples per day over a period of time have a serum cholesterol-reducing effect in humans. This agrees with work in Italy (9) which showed that eating the pulp of two or three apples after each meal reduced cholesterol levels. It can be argued that because people ate apples they consumed less of other items, thus producing an indirect effect by displacement of other dietary elements contributing to serum cholesterol concentration.

The trend found in this experiment was apparent after the subjects were on the apple diet for three weeks (Fig. 1, December 9). The eating patterns over Christmas may be responsible for the closeness of the serum cholesterol values for the (+) and (-) apple groups in January, while the difference re-asserted itself and became significant at February 17 and March 10 analyses dates. The apparent non-significant reversal in group serum cholesterol values in the three weeks after the apple diet terminated is interesting but a similar type of reversal or "rebound" phenomenon is commonly found in biological experiments. The rise in serum cholesterol values of both groups in the December, January period was attributed to a seasonal effect (14).

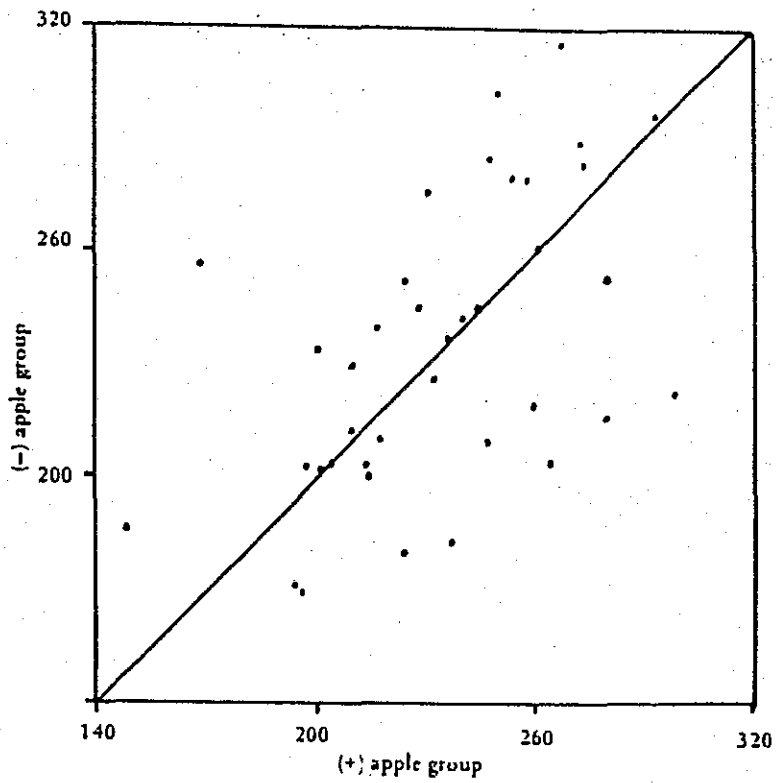


Fig. 2c: Scatter diagram for individual serum cholesterol levels (mg/100 ml) January 13

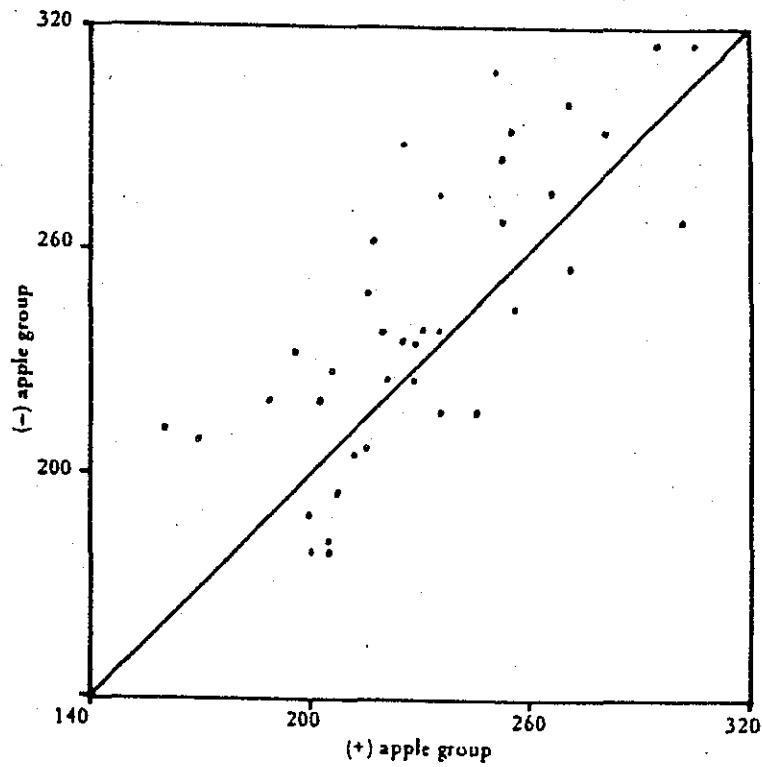


Fig. 2d: Scatter diagram for individual serum cholesterol levels (mg/100 ml) February 17

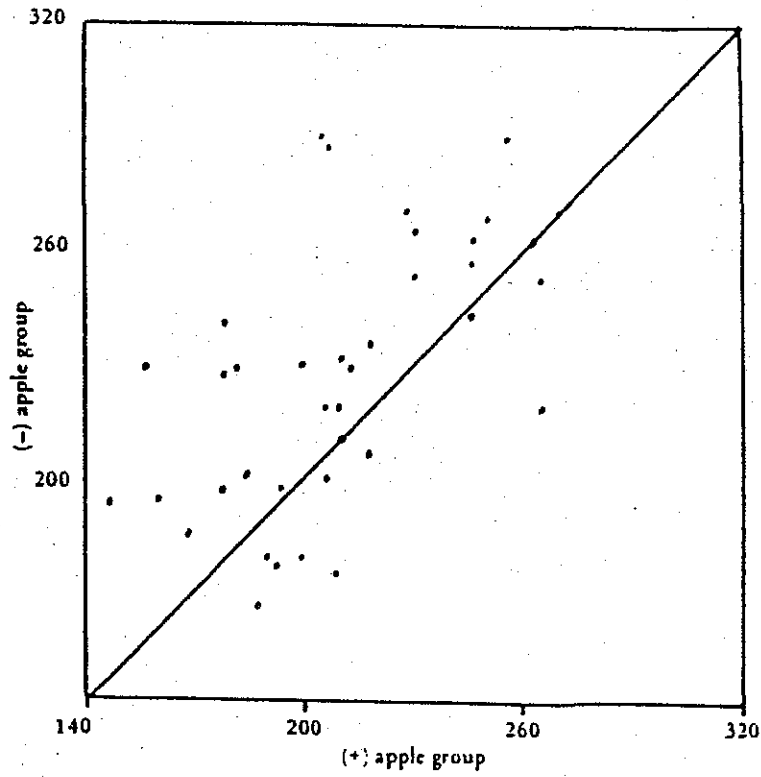


Fig. 2e: Scatter diagram for individual serum cholesterol levels (mg/100 ml) March 10

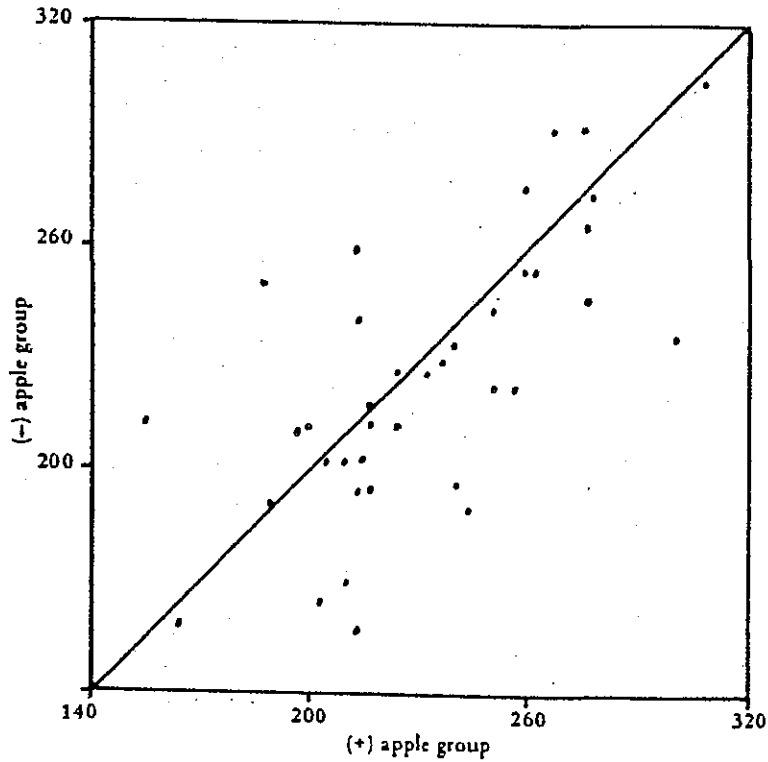


Fig. 2f: Scatter diagram for individual serum cholesterol levels (mg/100 ml) March 31

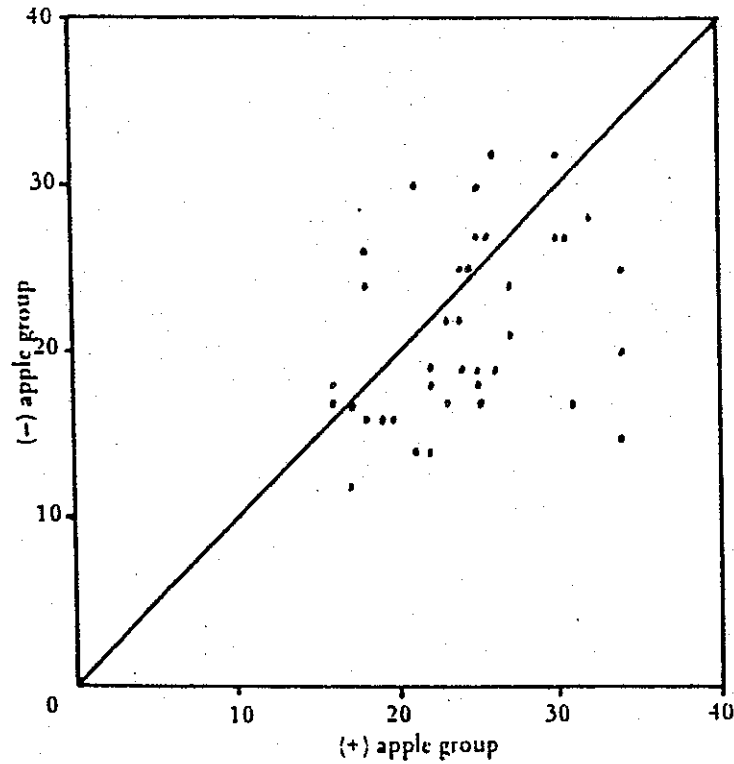


Fig. 3a: Scatter diagram for individual high density lipoprotein cholesterol levels (%)
March 10

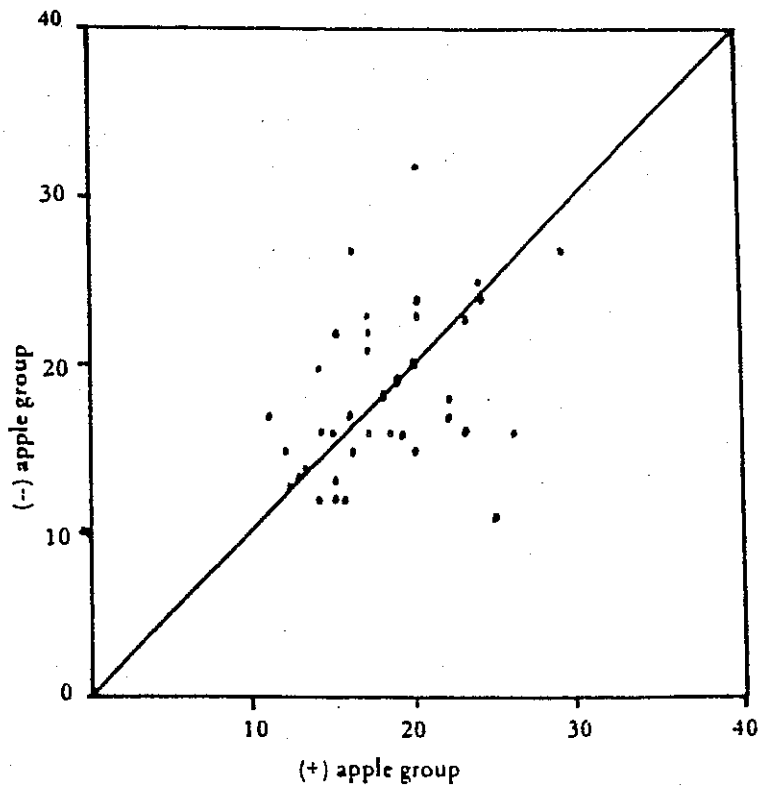


Fig. 3b: Scatter diagram for individual high density lipoprotein cholesterol levels (%)
March 31

While the difference in serum cholesterol values between the (+) and (-) apple groups was not large — 8.1% at maximum — the results suggest possible applications in programmes designed to reduce serum cholesterol levels on a community basis or for specific sub-groups deemed to be at risk from associated clinical events. These results must be viewed in the light that this reduction was achieved in subjects with relatively low serum cholesterol levels and by the increase of a single dietary component. Probably more pronounced effects could be produced in sub-groups with high serum cholesterol levels and by increasing dietary intake of other fruit and vegetable sources of dietary fibre, in addition to apples. A study is presently planned to test this concept in hyperlipidaemic subjects identified in screening programmes.

The apparent ability of apples to reduce serum cholesterol levels may be due to their pectin fraction and it is possible that a similar effect might be obtained with other fruits and possibly vegetables. Administration of large doses of pectin has been shown to reduce serum cholesterol levels (5).

In the context of long-term modification of dietary patterns in relation to heart disease risk it is possible that educational programmes emphasising a positive approach, involving increased intake of fruit and vegetables, might be more successful than traditional negative approaches recommending decreased intake of fat and protein.

The percentage of total cholesterol in the HDLP form was greater for the (+) apple group at the March 10 analysis date. Unfortunately % HDLP cholesterol was not tested earlier. It has been shown recently (15) that a high % HDLP cholesterol may represent a safety mechanism in the human system whereby cholesterol is returned to the liver rather than deposited in the tissues.

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