

# CHOLESTEROL METABOLISM AND ITS IMPLICATIONS IN MAN

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## I. INTRODUCTION

It is generally, if not quite universally accepted that hypercholesterolemia and triglyceridemia are indicators and risk factors for coronary heart disease (see for example Shaper 1972, Vergroesen 1972).

There is now a considerable literature beginning with the correlative observations of Walker and Arvidsson (1954) showing that the inclusion of fibrous materials in the diet may decrease plasma cholesterol levels. Trowell (1972) and Story and Kritchkevsky (1976) have extensively reviewed this literature. As might be expected not all sources and components of fibre are equally effective. Rolled oats (De Groot, Luyken & Pikaar, 1963; Luyken *et al.* 1962) and leguminous seeds (Anderson & Keys, 1965; Mather, Khan & Sharma 1968) have been shown to reduce blood cholesterol concentrations. Eastwood and co-workers (1973), Heaton and Pomare (1974) and Durrington, Wicks and Heaton (1975) obtained nil effects on cholesterol levels from the addition of wheat bran although Eastwood (1969) and Persson, Raby, Fønns-Bech and Jeason (1975) have reported significant falls.

As recommendations to increase fibre intake as a preventative and therapeutic measure against low-fibre associated diseases are usually met by wheat bran used as a supplement and because this is such a variable product depending on grain sources and milling practice, it was felt worthwhile to examine the effects of increased wheat bran intake on blood cholesterol levels in humans under local Australian conditions.

## II. MATERIALS AND METHODS

There were three experiments, all involved volunteers of both sexes mainly from the University of New England. A venous blood sample (5 ml) was withdrawn following an overnight fast to give the initial cholesterol concentration. Subsequent samples were taken at suitable intervals. Plasma was removed from whole blood following centrifugation and stored at  $-22^{\circ}\text{C}$  for cholesterol analysis using an enzymatic method (Calbiochem) in a Centrifcem System 400.

### (i) Experiment I

Twelve subjects (5 males and 7 females), all but one aged less than 25 years, were asked not to change their usual dietary habits but to take thrice daily, for 2 weeks, 3 bran snacks (Hygienic Food Supplies, West Ryde, N.S.W.) that contributed 2 g dietary fibre per day. Two subjects took instead 2 Bran Mini-Biscuits (Sanatarium Health Food Co., Sydney, N.S.W.) thrice daily; these also provided approximately the 2 g of dietary fibre. Blood samples were taken at the start of the experiment and again two weeks later.

### (ii) Experiment 2

Seventeen volunteers (12 females and 5 males) aged less than 40

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years were divided into two groups on the basis of an initial plasma cholesterol determination. Six subjects with a plasma cholesterol level below  $6.2 \text{ mmol l}^{-1}$  were asked to take foods known to be naturally high in cholesterol. These were largely dairy products, seafoods and some meats. The remainder (11) was asked to avoid these foods as far as possible. After three weeks on the dietary regimens all subjects took 12 g/d of wheat bran in equal amounts at each meal until the end of week 5. Both groups then reverted to their pre-experimental dietary habits. Venous blood samples were taken after 1, 3, 5 and 12 weeks. Diaries were kept by volunteers of foods consumed each day.

### (iii) Experiment 3

Seventy three (51 males and 22 females) volunteers aged from 20 to 57 years were asked to continue their normal diet and eating pattern. With the exception of 6 control subjects, all were told to add 5-6 g of dietary fibre each day as wheat bran. This could be taken in several different food combinations, e.g. wholemeal flour, Ryvita biscuits, wholemeal bread, certain breakfast cereals, bran muffins, etc. A blood sample was taken at the start and after 3 weeks on the regimen.

## III. RESULTS

### (i) Experiment I

The mean plasma cholesterol at the start and 2 weeks later was  $7.17 \pm 0.38$  and  $6.41 \pm 0.39 \text{ mmol l}^{-1}$  respectively. The reduction was significant ( $P < 0.01$ ).

### (ii) Experiment 2

The effect of diet was important in changing plasma cholesterol concentrations. (Figure 1). However, for the group with an initially high value, dietary change was apparently unable to reduce plasma cholesterol further after 1 week. The consumption of 12 g of wheat bran had an overriding influence in that plasma cholesterol of both groups declined significantly ( $P < 0.05$ ) at the end of week 5. When told to resume their normal dietary habits, those that had a naturally low cholesterol remained at this level, while those with an elevated cholesterol showed a significant increase at the end of week 12 compared with the mean value at week 5.

### (iii) Experiment 3

Data are from 65 volunteers who had an initial cholesterol concentration of  $5.9 \text{ mmol l}^{-1}$ , and  $5.8 \text{ mmol l}^{-1}$  after 3 weeks during which time they consumed an additional 5-6 g/d of dietary fibre. Those with an initial concentration of  $6.5 \text{ mmol l}^{-1}$  and above had a mean concentration of  $7.2 \text{ mmol l}^{-1}$  ( $N = 18$ ); this was reduced significantly ( $P < 0.01$ ) to  $6.6 \text{ mmol l}^{-1}$  at the end of 3 weeks. The six subjects used as controls showed no change ( $P < 0.05$ ) during the 3-week period.

## IV. DISCUSSION

Results of the first two experiments clearly demonstrate the ability of wheat fibre to reduce plasma cholesterol but only when the

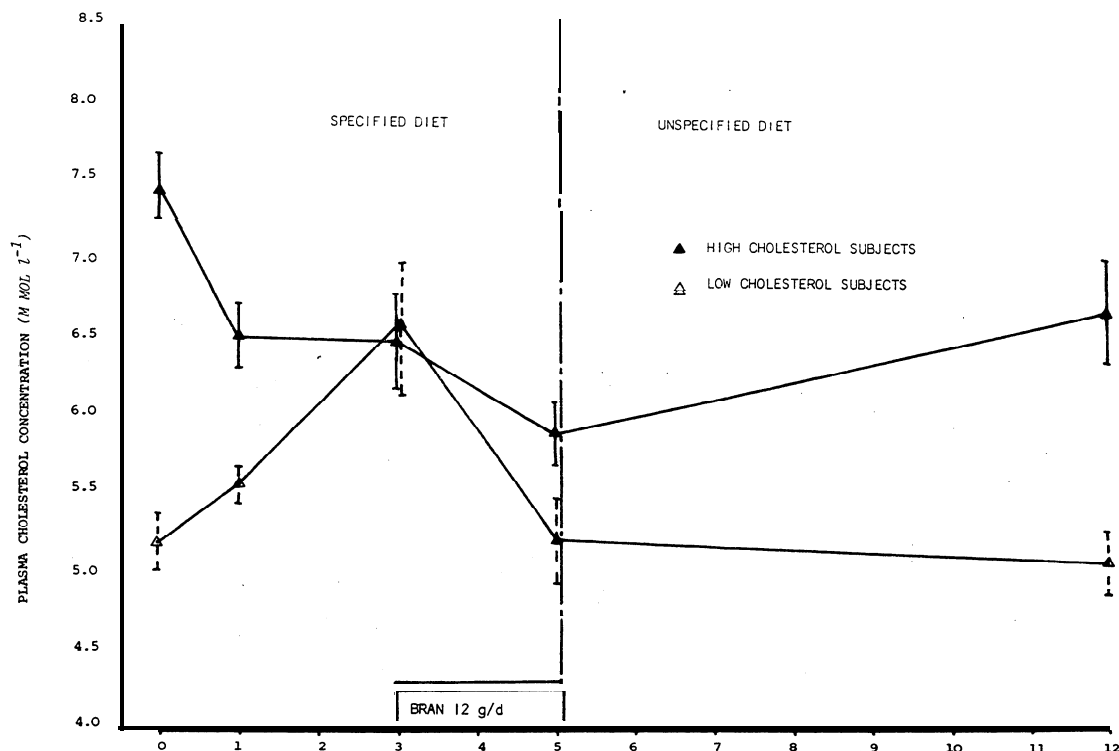


Figure 1. The cholesterol concentrations of 7 A and 11 A subjects with an initial low and high plasma cholesterol, respectively. Wheat bran was introduced, thrice daily, for 2 weeks when subjects had been on low  $\blacktriangle$  and high cholesterol A diets for 3 weeks. At the end of 5 weeks and onwards diet was unspecified.

concentrations could be considered above the norm (6.2 mmol l<sup>-1</sup>). In Experiment 3, the unusually low average concentration of 5.9 mmol l<sup>-1</sup>, coupled with the fact that many volunteers were already routinely taking considerable amounts of dietary fibre, as determined by a dietary survey of this group, indicates that the fibre addition was ineffective. However, bran was effective in lowering plasma cholesterol of those subjects that had a slightly elevated level at the start of Experiment 3.

Fibre appears to exert its effects on cholesterol levels not by interfering with the absorption of dietary cholesterol but by increasing turnover (Balmer & Zilversmit, 1974). The most likely point for intervention is the enterohepatic circulation of bile salts. In man some 30-40 mg of bile salts enter the gut daily of which only about 0.8 g escape in the faeces and have to be replaced by synthesis from cholesterol (Bergstrom & Danielsson, 1968). The simplest hypothesis for the fibre effect of increasing turnover and decreasing cholesterol level would be that binding of bile salts and acids to fibre prevents reabsorption, necessitating increased conversion of cholesterol to bile acids.

There is an appreciable amount of evidence supporting a mechanism of this kind. Animal experiments on fibre inclusion have shown increased faecal excretion of bile acids and sterols (Coleman and Baumann,

1957). Feeding of anion exchangers such as MK 135 (Bergen et al. 1959) . cholestyramine (Hashim & Van Itallie 1965) and colestipol (Parkinson et al. 1970) have all been shown to effectively lower plasma cholesterol concentrations. Eastwood and Boyd (1967) found appreciable quantities of bile acids bound to nonabsorbable materials in the small intestine.

Studies in vitro have confirmed the ability of dietary components to bind bile salts (Birkner & Kern 1974, Kritchnevsky & Story 1974 & 1975; Balmer & Zilversmit 1974). Story and Kritchnevsky (1975) made comparative studies of bile acids and bile salts to bran, cellulose and lignin. Adsorption to cellulose was negligible but lignin was highly effective as an adsorbent. The adsorption to bran was higher than would be accounted for by its lignin content and the pattern of adsorption was different. The ratio of cholate to chenodeoxycholate adsorption was about 0.5 for bran but about 2 for lignin. Taurochenocholate and glycochenocholate were also much more strongly adsorbed to bran than the corresponding cholate or deoxycholate derivatives. On these properties one would expect the chenodeoxycholate pool to be the most depleted when bran is fed.

In experiments in vivo Heaton (1972) found that the chenodeoxycholate pool fell on bran feeding. There are a number of possible explanations for this paradoxical finding. Deoxycholate is a secondary bile acid requiring microbial activity for its formation. Pomare and Heaton (1974) have found the 7- $\alpha$  dehydroxylase activity to be depressed by bran feeding. This coupled with the diminished transit time caused by bran feeding would appreciably decrease the rate of formation of deoxycholate. Finally there is appreciable microbial digestion of structural carbohydrates of fibre (Williams & Olmsted 1936; Hummel, Shepherd & Macy 1943; Milton-Thompson & Lewis 1971; Southgate & Durnin 1970; Southgate et al., 1976) so that binding at the site of reabsorption of the bile salts may be determined largely by the lignin (deoxycholate preferring) residue. It would be interesting to have information on the relative effectiveness of cholate, deoxycholate and chenodeoxycholate and their derivatives in the negative feed back control of  $\beta$ -hydroxy  $\beta$ -methyl-glutarylCoA reductase, the rate-controlling enzyme of cholesterol biosynthesis and cholesterol 7 $\alpha$ -hydroxylase, the rate limiting enzyme in bile acid formation.

There are a number of unsatisfactory features in the simple bile salt binding hypothesis. Lignin which binds in vitro has been shown to be ineffective in vivo (Heaton & Barry 1971). The chemistry of the binding of bile salts by fibre is obscure. Oakenfull (1977) examined a range of purified fibres and fibre components and found binding only with lucerne and soya bean. The binding in these he attributed to the presence of saponins.

Bile salts might bind ionically to fixed cations or hydrophobically to non-polar materials. In the common bile salts there are at least two hydroxyls, the carbonyl and the -NH- group of the amide link as well as the carboxylate or sulphonate groups capable of hydrogen bonding. H bonds are weak and also directional so that multiple bonding on a fixed carbohydrate matrix is unlikely. Eastwood and Mitchell (1976) found that although most natural fibre residues had appreciable cation exchange capacity they were unable to detect any anion exchange properties. This is perhaps not too surprising since no aminosugars have been detected in plant cell wall polysaccharides.

The ability to sequester bile salts may be linked to the gel-forming potential of the fibre. (Ershoff & Wells 1962). Pectin (Fisher et al. 1965; Anderson, Grande & Keys 1973); Jenkins et al. 1975) and Guar gum (Fahrenbach et al. 1965; Jenkins et al. 1975) have both been shown to be effective in lowering cholesterol levels in man. There is a wealth of evidence showing that pectin is particularly effective in preventing the hypercholesterolemia induced in animals by cholesterol feeding and may lead to increased faecal excretion of bile acids and sterols (Fisher et al. 1966; Lin et al. 1967). An observation indicating a more complex mechanism than simple sequestration of cholesterol and bile salts is the reduction observed in the biosynthesis of liver cholesterol (Moday 1974). Recently guar gum has come into prominence as a possible means of controlling mild diabetes due to its effect in slowing glucose absorption. Insulin promotes hepatic lipogenesis and the synthesis triglyceride rich circulating lipoproteins (Faraahar et al. 1966); it is also one of the hormones involved in the induction of  $\beta$ -hydroxy  $\beta$ -methyl-glutaryl CoA reductase (EC1.1.1.34). A slower rate of glucose entry might be expected to lead to diminished insulin secretion.

It is clear that a great deal more information is required before the relative importance of the various mechanisms proposed for the lowering of blood cholesterol, bile salt binding with increased drainage from the cholesterol pool, altered feed back control due changes in the proportions of the bile acids or altered hormonal control due to changes in rate and quantity of absorption of glucose and other metabolites, can be determined. There is a need for more detailed investigation in these areas but also a need to widen the range of parameters measured in experiments on fibre for the true explanation for the effects may lie in an area as yet unexplored. The simple assay of plasma cholesterol in experiments of this type needs to be supplemented by determination of high and low density lipoproteins and by glucose and insulin tolerance tests.

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