THE AUSTRALIAN POLYP PREVENTION PROJECT: DESIGN, FOLLOW-UP, AND PRELIMINARY ESTIMATES OF DIETARY COMPLIANCE

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Summary

Betacarotene (20mg capsule daily v placebo), a low-fat diet (≤25% energy as fat v no change) and added fibre (25g wheat bran supplement daily v none) were tested as preventives of adenomas of the large bowel in a 2x2x2 factorial design. Of 424 patients in three cities 96% were followed for two years. Compliance with all regimens was excellent: 90% for betacarotene (pill count), 92% for fat reduction (24-hour recall) and 83% for fibre supplements (24-hour recall) at two years (Brisbane data, n=122). Diet diaries confirmed these findings. Serum betacarotene was increased eight-fold in those on active drug (v no change with placebo); fat consumption decreased 8.3% (v 5.3% in controls); and fibre intake increased 41.2% (v no change in controls). Thus power to detect effects of the interventions remains high.

I. INTRODUCTION

Attempts to identify modifiable dietary causes of colorectal cancer (CRC) have had limited success (Wahrendorf 1987). Difficulties associated with observational studies, particularly misclassification of diet, have led to variable results; and direct experimental approaches to the issue with cancer incidence as the end point remain unattractive (Zelen 1988). Magnus and Miller (1980) have pointed out advantages of using pre-cancerous lesions as end points, including shorter follow-up and possibly higher power to detect an effect.

Thus, while adenocarcinoma would be the most relevant and valid end point in a trial to prevent CRC, such a trial would require some 40,000 subjects followed over very many years. Short-term end points such as intestinal cell proliferation are feasible but of uncertain relevance to cancer prevention (Lippman et al. 1990). For our trial, the Australian Polyp Prevention Project (APPP), then, precursor adenomas were chosen as the outcome for study. They are common, follow-up colonoscopy is routine clinically and new adenomas have a high cumulative incidence. There is also

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good evidence for adenomas being the usual precursors of CRC (Morson 1974; Aitken 1990).

(a) Choice of preventive measures

When planning the APPP (1982 to 1984), preventive measures considered were fat reduction; increased dietary fibre, fruits and vegetables; and supplements of betacarotene, retinol, calcium or selenium. Following extensive literature review, the interim dietary guidelines of the US National Research Council's Committee on Diet, Nutrition and Cancer (1982) recommended reduced fat intake for the prevention of CRC. An appropriate practical target was to reduce fat intake from about 40% to 30% of total energy. On this basis fat reduction was included as an intervention in our trial.

Populations with low colorectal cancer risk generally have high intakes of dietary fibre. A review by Willett and MacMahon (1984), supported recently by others (Freudenheim and Graham 1989; Trock et al. 1990), indicated that although the available epidemiologic data were not entirely consistent, the weight of evidence favoured fibre protecting against CRC. There is some difficulty in interpreting simple relations between fibre intake and cancer because foods high in fibre (particularly vegetables) contain other substances that may be preventive. Hence we increased cereal fibre intake with a supplement rather than using primary food sources.

Several epidemiological investigations have suggested a link between low "vitamin A" intake and increased risk of a variety of cancers. "Vitamin A" refers to both ingested vitamin A and betacarotene, a provitamin that may be converted to vitamin A in vivo. Low retinol in stored sera has been associated with subsequent increased cancer risk, but CRCs were too few to estimate risk at this site (Kark et al. 1980). A significant inverse association was found with a heterogeneous group of gastrointestinal cancers (Willett et al. 1984). Questionnaire studies of cancer in relation to intake of some betacarotene-rich vegetables or of "vitamin A" have been reviewed by Peto et al (1981) who found that use of certain vegetables rich in betacarotene was associated with a reduced risk of CRC. Among the various sources of "vitamin A" activity, betacarotene was chosen as being minimally toxic with a good likelihood of being efficacious.

Increasing fruits and vegetables was considered as an intervention but could not answer directly questions of nutrient contributions to prevention; information on the effects of calcium was limited; and selenium and retinol were considered potentially toxic.

II. METHODS

(a) Trial design and interventions

Interventions were selected the better to test specific hypotheses, realising that if protective effects were identified, community implementation could well require different strategies. To maximise efficiency we used a 2x2x2 randomised factorial design (Freedman and Green 1990). Fat was reduced to at least 30% of total energy, and to 25% if feasible, versus unaltered intake; a fibre supplement of 25g wheat bran daily (giving 11g dietary fibre) was provided, versus unaltered intake; and betacarotene was administered (from a calendar pack) as a daily 20 mg capsule versus placebo capsule. Thus in those taking betacarotene a quarter of subjects were on reduced fat intake, a quarter on added bran, a quarter on both reduced fat and added bran, and a quarter had no recommended dietary change. The same was true for those taking placebo capsules, giving eight groups in all.

(b) Sample size

The main objectives of the trial require independent estimation of rates of new adenomas under the three regimens, with a 50% reduction considered clinically important. The expected proportion of subjects with new lesions after two years follow-up was 40%. This reflected clinical experience in participating Australian centres and is approximately the rate reported by McKeown-Eyssen et al. (1988) from Toronto. With a type I error rate of 5% and type II of 1% a minimum total sample of 422 (211 on each treatment) would be required to detect a 50% reduction in rates of new adenomas (Schlesselman 1982). Were a one-sided test used, this number is 324. A decrease of only 30% would require larger numbers to maintain the same power: with the same sample size the power would be almost 90%. A lower occurrence rate of new lesions also reduces power: if the rate were 20% (and 10% in an intervention group), the power would be 80%. To obtain such a number reasonably rapidly, patients were enrolled simultaneously in three cities (Brisbane, Sydney, Melbourne). The first participant was enrolled in October, 1985 and the last in May, 1988. Given the planned two year follow-up, 24-month surveillance was due to finish in May, 1990.

(c) Selection of patients

Eligibility criteria for patients were age (over 30 and under 75 years); a "clean" large bowel following colonoscopy; histological verification of at least one adenoma; and signed informed consent. Patients were excluded if they had chronic inflammatory bowel disease, gastrointestinal tract resection, familial adenomatous polyposis, previous cancer unless symptom free for five years, or were on medical diets. Additional exclusions reflected clinical judgement that the patient was unlikely to complete the trial for medical or other reasons, or comprehended English poorly. Collaborating centres needed to have at least 40 new eligible patients per year with participating clinicians certified by the Gastroenterlogical Society of Australia ceritifed and performing at least 4 colonoscopies per week to reduce the likelihood of misclassification of outcomes.

(d) Randomisation

Randomisation objectively distributes both known and unknown prognostic factors among treatment groups, but not necessarily evenly. To help improve balance, we used pre-randomisation stratification (Peto et al. 1977). Eligible patients were stratified by city, surveillance status at entry (new patient or follow-up) and age (<55, >=55 years), and then randomised to treatment groups by the coordinating centre. Randomisation within strata was performed in blocks, so that the treatment assignments were exactly balanced among every eight allocations.

(e) Assessment of compliance

To assess properly the results of prevention trials, it is desirable to know the extent to which participants have utilised each preventive and the extent to which controls spontaneously changed relevant behaviours (Freedman 1990). Here compliance was assessed primarily by participants' self-report to dietitians and research nurses. Some more objective measures were also included.

The dietitians and research nurses kept in close contact with patients throughout the trial by telephone and in person, thus maintaining motivation. Dietitians collected information with 24-hour dietary recalls every three months, and based their dietary counselling on this. Unused capsule and fibre supplement counts were made at the same time. In addition, a more objective measure of compliance was built in at six-monthly follow-ups by the nurse, who independently administered four-day estimated food records.

Biochemical measures of compliance were made on blood samples taken initially and every six-months. They included betacarotene, retinol and cholesterol. Betacarotene was measured in the Conjoint Internal Medicine Laboratory of the Royal Brisbane Hospital by a modification of the method of Milne and Botnen (1986). Plasma cholesterol assays were performed at the Monash Medical Centre according to a standard enzymatic method (Allain et al. 1974). Other markers of compliance investigated for their utility were breath hydrogen and plasma acetate as measures of fibre intake; red cell and plasma fatty acids as a measure of reduced fat; and urinary oestrogens as a measure of fibre intake and/or reduced fat intake.

(f) Assessment of outcome

Surveillance colonoscopy was planned to occur after 2 years in all subjects. Earlier examination occurred occasionally where clinically indicated. Sites of the large bowel inspected at colonoscopy were recorded together with the location, number and size of adenomas and other pathology. All polyps had histopathology assessed according to the WHO protocol (Morson and Sobin 1976) and degrees of villosity and dysplasia were scored. Uniform review of histology was performed.

(g) Data analysis

Recruitment and follow-up data are presented for the whole trial. Baseline and compliance data are given for Brisbane patients only, as data entry and validation are not yet complete for other centres. Food intake data were analysed with Nutritionist III (N-Squared Computing, 1986). The nutrient data base was modified for dietary fibre with values from Paul and Southgate (1978), supplemented by data for Australian foods (supplied by Department of Human Nutrition, Deakin University), subsequently incorporated in NUTTAB (Department of Community Services and Health 1988). The values for fat are not identical with recent values for Australian foods, so relative intakes will have higher validity than absolute intakes. A repeated measures analysis of variance was used to compare changes in fat and fibre intake over time.

II. RESULTS

(a) Recruitment and follow-up

Recruitment closed when 424 patients were enrolled in the trial (76% of those eligible). The overall numbers screened for eligibility are shown in Table 1. Of the post-colonoscopy exclusions, almost half (364) had no adenoma confirmed and 29% (219) had other bowel disease, including CRC.

Table 1. Recruitment to the APPP

Category	Number	(%)
Total polyp patients registered	2780	(100)
Excluded pre-colonoscopy	1476	(53.1)
Excluded post-colonoscopy	745	(26.8)
Total eligible	559	(20.1)
Refusals	135	(4.9)
Enrolled patients	424	(15.3)

Each city contributed similar numbers: Melbourne 159, Sydney 143, Brisbane 122. Even balance was achieved in the different arms of the trial, with numbers included in the eight groups ranging from 51 to 56.

Follow-up has been good, with 96% of patients (406) having the presence or absence of adenomas of the colorectum ascertained after two years on

trial. Rates were similar for each city. Of the remaining 18, 2 had earlier colonoscopies on clinical grounds and 3 were inadequately reviewed.

(b) Baseline characteristics

The distribution of various features of patients randomised in Brisbane to betacarotene and placebo are compared in Table 2.

Table 2. Distribution of baseline characteristics for Brisbane patients according to intervention status[1]

Variable	Randomisation group	
	Intervention	Control
Mean age (years)	56.0 (10.8)[2]	55.0 (9.7)
% male	62.9	61.7
% first adenoma (v subsequent)	83.9	85.0
Mean no. adenomas (entry)	1.21 (0.55)	1.10 (0.30)

1 data shown for betacarotene (n=62) and placebo (n=60) groups; distributions were similar for the other interventions
2 (standard deviation, sd)

(c) Compliance

Estimates of compliance for each of the three interventions, over two years of follow-up, are shown in Table 3. These are the working estimates used by the dietitians for counselling purposes.

Table 3. Compliance (%) in APPP: dietitians' working estimates for Brisbane patients

Follow-up interval (months)	Intervention		
	Betacarotene[1]	Fat reduction[2]	Fibre supplementation[3]
6	93 (18)[4]	87 (15)	87 (27)
12	93 (20)	86 (18)	85 (28)
18	93 (19)	91 (14)	83 (31)
24	90 (24)	92 (15)	83 (30)

1 capsule counts (those for placebo were very similar)
2 100% compliance achieved if fat intake at follow-up was ≤ 25% total energy intake at baseline (based on 24 hr recalls)
3 dietitians' estimates of use of supplement
4 (sd)

Differences in compliance among subgroups of sex, age, year of entry and so forth were generally very small. Placebo capsules were taken at the same high rate as active betacarotene.

Confirmation of capsule counts was sought by measuring serum betacarotene. Baseline and twelve month values in Brisbane again indicate good compliance. Whereas mean baseline levels (sd) were similar in the two groups - 298 (198) and 269 (169) μg/L for intervention and placebo respectively - after one year the means were 2389 (1419) and 272 (198) μg/L, an eight-fold increase among those taking the active drug versus no change. Estimated "compliance", defined as achieving a 12-month value at least three standard deviations above the baseline mean, was achieved by 89%.

Dietitians' estimates of compliance with the low fat regimen were validated against measures based on four-day diet diaries concurrently administered by research nurses. Changes in plasma cholesterol were also monitored. Measurements of red cell and plasma fatty acids were abandoned after pilot studies showed no correlation with changes in diet.

Table 4. Independent assessments of compliance with a low fat diet in the APPP (Brisbane)

Variable		Time period (months) 0	6	12	24
% compliance[1]	I[2]	-	87 (17)[3]	89 (15)	90 (14)
Fat intake (g)[4]	I[2]	71 (27)	57 (21)	57 (20)	58 (22)
	C[2]	76 (25)	75 (29)	72 (28)	72 (25)
Fat intake	I	31 (5.6)	27 (7.0)	27 (6.2)	28 (6.2)
(% energy)[4]	C	34 (6.2)	34 (10)	33 (7.4)	33 (9.4)
Megajoules	I	8.6 (2.7)	7.7 (2.5)	7.7 (2.6)	7.7 (2.6)
	C	8.4 (2.6)	8.1 (3.0)	8.0 (3.0)	7.8 (3.5)
Plasma choles-	I	6.3 (3.6)	5.9 (1.5)	6.4 (2.2)	5.7 (1.7)
terol[5](mmol/L)	C	6.4 (1.3)	6.2 (1.8)	6.5 (1.3)	6.3 (1.8)

1 compliance estimated as in Table 4, but based on 4-day diaries
2 I = intervention (low fat), n=61; C=control, n=61
3 (sd)
4 group means from 4-day diaries
5 n=28 (intervention), 23 (control)

Absolute fat intake in the intervention group was 19.7% lower than baseline at six and 12 months and still 18.3% less at two years (all significant at P<.001). Control intake was reduced slightly (by 5.3% at one and two years). As total energy consumption also declined during the study, changes in fat as a proportion of energy were somewhat less striking (-12.9% at 12 months and -9.7% after 2 years on lowered fat and -2.9% at the same points among controls). Among a small sample plasma cholesterol was 9.5% lower at two years than baseline among those on reduced fat, but had been close to baseline at one year. Control values were fairly constant.

The proposed objective measures of fibre consumption - breath hydrogen and plasma acetate - have so far proved infeasible as practical markers of compliance. Figures for actual fibre consumption are given in Table 5.

Table 5. Fibre consumption in the APPP (Brisbane)

Measure of total fibre (g)		Time period (months) 0	6	12	24
4-day diary	I[1]	17 (7)[2]	23 (11)	24 (10)	24 (10)
	C[1]	17 (8)	18 (12)	16 (8)	17 (10)

1 I = intervention (added fibre), n = 58; C = control, n = 64
2 (sd)

Those adding fibre thus appeared to increase consumption and maintain it about 7 grams (41.2%) above baseline throughout (P<.001), compared to essentially no change in the comparison group (P>0.28).

III. DISCUSSION

There is always the possibility that a long-term preventive trial could be passé before it is over. Fortunately this does not seem to be true of the APPP. The general benefits of its interventions remain speculative, belief in the adenoma-cancer sequence continues strong (Aitken 1990), and the factorial design still seems best (Freedman and Green 1990).

The fate of a multicentre, multi-investigator intervention study rests with the enthusiasm of its members. In particular, the dedication of colonoscopists and their clinic staff have produced virtually complete

follow-up at two years, greatly enhancing the internal validity of the trial. This was further secured by apparently successful randomisation and the blinded, objective, clinically accurate outcome measure. Participants were also ignorant of their exposure to betacarotene. While no perfect placebo is available for dietary advice, those on capsules only (one-quarter) were contacted three-monthly by the research nurse; and all completed diet diaries and provided blood and urine samples at the same intervals. Thus results of the APPP should reflect biological reality for participants. As the principal issue in generalising such data is biological rather than statistical (in the sampling sense), the selected nature of the APPP sample does not restrict the external validity of the results. More limiting to public health applications may be the fairly intensive dietary counselling and support, which is unlikely to be practicable on a large scale.

Compliance over the two years was remarkably good. While the less-objective 'working' measures based on pill counts and dietitian-administered 24-hour dietary recalls are imperfect, their estimates of compliance rates at two years of 90% for taking betacarotene capsules and 92% and 83% for reaching dietary goals for fat reduction and fibre supplementation respectively, were supported by more objective measures.

Whereas capsule counts have been seen as less than ideal indicators of compliance in the clinical setting (Gordis 1979), recent evidence from other chemoprevention studies, including use of a riboflavine marker (Moon 1986), is far more positive. Our own grouped data show pill count and serum betacarotene to give concordant estimates of compliance.

Dietitians' estimates of compliance (from 24-hour recalls) with the low fat regimen were mirrored by those derived from diet diaries, which have been shown by others to provide good measures of change (Henderson et al. 1990). Small numbers make the fluctuating levels of plasma cholesterol difficult to interpret. While good compliance was achieved, and fat consumption was significantly reduced, it may be that our goals for the intervention group could have been more extreme. The baseline values were lower than expected, and the Vanguard Study of the US Women's Health Trial has recently reported maintaining reduction in daily fat intake over two years to a mean of 34.1 grams, or only 22.6% of energy (Henderson et al. 1990). It remains to be seen if the more modest achievement in the APPP will be sufficient to alter adenoma incidence.

For fibre, dietitians' estimates of compliance have not been confirmed so securely, given the lack of an objective marker or external validation of the diary measurements of intake. Nonetheless, the latter recorded a pattern of substantial and persisting increase in reported fibre intake by the supplemented group. There is, therefore, good reason to presume that losses of power due to noncompliance - a major issue in long term interventions (Freedman 1990) - have been minimised, giving a good chance of detecting true beneficial effects of the measures being tested.

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REFERENCES

AITKEN, J.A. (1990). PhD Thesis, University of Queensland.

ALLAIN, C.C., POON L.S. and CHAN, C.J.G. (1974). Clin. Chem. 20: 470.

DEPARTMENT OF COMMUNITY SERVICES and HEALTH. (1988). 'NUTTAB'. (AGPS: Canberra).

FREEDMAN, L.S. (1990). Controlled Clin. Trials 11: 157.

FREEDMAN, L.S. and GREEN, S.B. (1990). J. Natl. Cancer Inst. 82: 910.

FREUDENHEIM, J.L. and GRAHAM, S. (1989). Epidemiol. Rev. 11: 229.

GORDIS, L. (1979). In 'Compliance in Health Care', p.23, eds R.B. Haynes, D.W. Taylor and D.L. Sackett. (Johns Hopkins Press: Baltimore).

HENDERSON, M.M., KUSHI, L.H., THOMPSON, D.J., GORBACH, S.L., CLIFFORD, C.K., INSULL, W., MOSKOWITZ, M. and THOMPSON, R.S. (1990). Prev. Med. 19: 115.

KARK, J.D., SMITH, A.H. and HAMES, C.G. (1980). J. Chron. Dis. 33: 311.

LIPPMAN, S.M., LEE, J.S., LOTAN, R., HITTELMAN. W., WARGOVICH. M.J. and HONG, W.K. (1990). J. Natl. Cancer Inst. 82: 555.

MAGNUS, K. and MILLER, A.B. (1980). J. Natl. Cancer Inst. 64: 693.

McKEOWN-EYSSEN, G., HOLLOWAY, C., JAZMAJI, V., BRIGHT-SEE, E., DION, P. and BRUCE, W.R. (1988). Cancer Res. 48: 4701.

MILNE, D.B. and BOTNEN, J. (1986). Clin. Chem. 32: 874.

MOON, T.E. (1986). Stats. Med. 5: 435.

MORSON, B.C. (1974). Cancer 34: 845.

MORSON, B.C. and SOBIN, C.H. (1976). 'Histological Typing of Intestinal Tumours'. International Histological Classification of Tumours No. 15. (W.H.O.: Geneva).

NATIONAL RESEARCH COUNCIL. (1982). Diet, Nutrition and Cancer. (National Academy Press: Washington).

N-SQUARED COMPUTING. (1986). 'Nutritionist III'. (N-Squared Computing: Silverton).

PAUL, A.A. and SOUTHGATE, D.A.T. (1978). 'McCance and Widdowson's The Composition of Foods', 4th edn. (HMSO: London).

PETO, R., DOLL, R., BUCKLEY, J.D. and SPORN, M.B. (1981). Nature 290: 201.

PETO, R., PIKE, M.C., ARMITAGE, P., BRESLOW, N.E., COX, D.R., HOWARD, S.V., MANTEL, N., McPHERSON, K., PETO, J. and SMITH, P.G. (1977). Br. J. Cancer 35: 1.

SCHLESSELMAN, J.J. (1982). 'Case-control Studies: Design, Conduct, Analysis', p. 145. (Oxford University Press: Oxford).

TROCK, B., LANZA, E. and GREENWALD, P. (1990). J. Natl. Cancer Inst. 82: 650.

WAHRENDORF, J. (1987). In 'Causation and Prevention of Colorectal Cancer', p.155, eds J. Faivre and M.J. Hill. (Elsevier Science Publishers: Amsterdam).

WILLETT, W.C. and MacMAHON, B. (1984). New Engl. J. Med. 310: 697.

WILLETT, W.C., POLK, B.F., UNDERWOOD, B., STAMPFER, M.J., PRESSEL, M.S., ROSNER. B., TAYLOR, J.O., SCHNEIDER, K. and HAMES, C.G. (1984). N. Engl. J. Med. 310: 430.

ZELEN, M. (1988). J. Natl. Cancer Inst. 80: 1278.