Chronic and postprandial responses of plasma insulin, glucose and lipids in volunteers given dietary fibre supplements

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We questioned whether a dietary fibre supplement known to lower fasting plasma cholesterol concentrations can also lower the postprandial plasma cholesterol, glucose and insulin concentrations when it is administered just before a meal. Two studies were conducted in healthy middle-aged volunteers of both sexes in whom the fasting plasma total cholesterol concentrations were above normal. In the first study the dietary fibre treatments (2·2 g) were psyllium and a psyllium–citrus pectin mixture to which the subjects (four males, eight females) had no prior exposure. Controls received no supplement. The meals were high-fat breakfasts and lunches. In the second study the dietary fibre (6 g) was from sugar-beet root and the reference control was α-cellulose (2 g); the meal was of glucose. The volunteers (eight males, eight females) had prior exposure to the fibre supplements three times daily for 3 weeks. After adjustments for fasting values and changes in haemodilution, the psyllium and psyllium–citrus pectin mixture in the first experiment had no significant effects on the postprandial measurements of plasma glucose, insulin:glucose ratio, total-, LDL- and HDL-cholesterol, and triacylglycerol. By contrast, the sugar-beet fibre in the second study significantly decreased the area under the glucose response curve by 6·9%, the area under the insulin response curve was lower by 9·6%, although not significantly, and the post-glucose meal HDL-cholesterol concentration was significantly (12%) higher. Additionally, the 3-week treatment with sugar-beet fibre significantly lowered the fasting total- and LDL-cholesterol concentrations, by 8·5% and 9·6% respectively. We conclude that low doses of psyllium and citrus pectin at breakfast and lunch have no effects on the postprandial plasma measurements, but that sugar-beet fibre taken daily for 3 weeks affects both fasting and postprandial plasma metabolites favourably in these individuals with mildly increased risk of ischaemic heart disease. Further, we observed that small changes in haemodilution occur after meals, as indicated by plasma albumin concentration and packed cell volume. Underemphasis of the dietary fibre effects may occur when postprandial haemodilution is not taken into account.

Dietary fibre: Cholesterol: Insulin: Postprandial lipaemia: Human

An excessive intake of dietary fat is considered to be a causal agent of ischaemic heart disease (IHD) and the risk of this is recognized to be positively correlated with fasting serum total cholesterol (Pocock *et al.* 1989; Pekkanen *et al.* 1990) and low-density-lipoprotein (LDL)-cholesterol (Pekkanen *et al.* 1990) concentrations. The absorption and transport of dietary fat is mediated by plasma lipoproteins and those derived in the intestines are implicated in atherogenesis. An understanding of this may be advanced by investigations into lipoprotein metabolism in the fed, rather than fasted, state (Cohn *et al.* 1988). Several studies into postprandial (PP) changes in lipoprotein fractions have employed young volunteers with low fasting concentrations of plasma cholesterol (Patsch

et al. 1983; Groot & Scheek, 1984; Cohn *et al.* 1988) which were below a proposed lower limit of 5·15 mmol/l for significant IHD risk (Pekkanen *et al.* 1990). Little work has been conducted on middle-aged subjects above this limit with slightly elevated triacylglycerol (TAG) concentrations. A high-fat diet and elevated plasma TAG induce insulin resistance and the resulting glucose intolerance is an IHD risk factor (Ohlson *et al.* 1989; Sidery *et al.* 1990; Simonson, 1990; Yudkin *et al.* 1990). Insulin stimulates cholesterol synthesis (Stinson *et al.* 1991; Tomkin & Owens, 1991) and impaired insulin action is associated with low concentrations of plasma high-density-lipoprotein (HDL)-cholesterol (Dean *et al.* 1991).

The chronic consumption of certain sources of water- soluble fibre lowers fasting plasma concentrations of cholesterol. A preprandial dose of guar gum (3×10 g/d) in water decreased fasting concentrations of blood glucose, cholesterol and TAG (Landin *et al.* 1992). Our purpose was to determine whether similar effects occurred postprandially. In two experiments in middle-aged volunteers with a mildly increased risk of IHD we determined the effects of psyllium, citrus pectin or sugar-beet fibre supplements on PP plasma metabolites and hormone responses. In the first experiment subjects received water-soluble fibre in tablet form before a breakfast and a lunch. In the second they received powdered fibre supplements before a glucose tolerance test at the end of a chronic study during which the supplements were taken before meals. Plasma concentrations of cholesterol and its fractions, total TAG, glucose and insulin were measured.

MATERIALS AND METHODS

In two studies dietary fibre supplements were given to adult volunteers who satisfied the entry requirements described in Expt 1. All supplements were commercially produced, purified fibre extracts of vegetable material. In a 4 d study (Expt 1) the control treatment was no supplement, so that subjects were aware of allocation to test treatments. In a 3-week study (Expt 2) subjects were unaware of the nature of the treatment.

Experiment 1

The purpose was to establish the pattern after breakfast and lunch of plasma lipid, glucose and insulin responses to fibre sources known to lower chronically the fasting plasma cholesterol concentration. Constant breakfast and lunch meals (Tables 1 and 2) were followed by a constant evening meal, so that an isoenergetic diet was provided for twelve subjects over 4 d using a complete set of three orthogonal 4×4 Latin squares (Jones & Kenward, 1989). The four treatments were: no supplement (control, D), or three types of supplement tablet, each containing 2·2 g water-soluble fibre, 1·0 g purified lecithin and 1·52 g natural apricot fruit flavours. The tablets were chewed and consumed over a period of 5 min immediately before breakfast and lunch. Treatment A contained purified psyllium, B contained 1·1 g psyllium and 1·1 g purified citrus pectin, and C was a repeat of treatment A. Treatments were given in a randomized order, consistent with the design, one treatment per day, over the 4 d.

Four males and eight females, mean 50·6 (SD 8·56) years of age (range 29 to 62 years), participated. Their mean body mass index (BMI) was 28·2 (SD 5·40) kg/m^2 and mean fasting plasma TAG and total cholesterol concentrations were 1·32 (SD 0·17) and 6·0 (SD 0·17) mmol/l respectively. They were admitted to the Clinical Investigation Unit at approximately 15.00 hours on day 1 for a medical screening and for taking the first controlled evening meal at 17.30 hours. All were free of significant medical disorders and none was undergoing treatment with any type of drug. On days 2 to 5 the subjects were admitted to the unit at approximately 07.30 hours and they left at approximately 18.00

Table 1. *Expt 1. Composition of meals (g/d per subject)*

Breakfast		Lunch	
Scrambled egg		Shepherd's Pie	
Whole egg	77·0	Minced steak	200·0
Potato	41·0	Vegetable puree	
Cheddar cheese	25·0	(green beans, carrot, cauliflower)	50·0
Double cream	5·6	Dried potato	34·0
Butter	6·6	Tomato	27·0
Salt and pepper	+	Kidney puree	25·0
Total	155·2	Cheddar cheese	20·0
Yoghurt	150·0	Onion	15·0
Orange juice	150·0	Butter	12·1
Water	+	Double cream	4·5
		Tomato puree	3·1
		Beef stock	2·3
		Egg yolk	2·2
		Maize starch	1·5
		Garlic	1·2
		Salt	+
		Black, white and red pepper	+
		Water	149·0
		Total	546.9
		Orange juice	150·0
		Meringue	
		Double cream	90·0
		Crushed meringue	14·0
		Stem ginger	3·0
		Water	+

Table 2. *Expt 1. Chemical composition of meals (amount per day per subject)*

	Breakfast	Lunch
Crude protein (g)	24·1	79·4
Total lipids (g)	36·0	86·7
Starch plus sugars (g)	21·3	61·0*
Energy		
Gross (MJ)	2·1	6·7
Metabolizable* (MJ)	2·4	5·5
Calcium (g)	0·41	0·26
Dietary fibre (g)	1·6	7·18
Fat constituents		
Total saturated fat (g)	17·9	47·4
Total monounsaturated fat (g)	14·2	35·2
C18:1 (g)	12·8	30·3
Total polyunsaturated fat (g)	3·8	3·9
C18:2 (g)	2·9	2·6
C18:3 (g)	0·58	0·87
C20:3 (g)	0·17	0·19
Cholesterol (g)	0·42	0·63

* Calculated from tables in Paul & Southgate (1978).

hours. On these days the first subject commenced breakfast at 08.32 hours and lunch at 13.02 hours daily. The other eleven subjects commenced eating in sequence at 2 min intervals after the first subject. The same sequence of eating occurred at both breakfast and lunch on each day. No physical exertion, other than walking, was permitted in the unit, no consumption of alcohol, coffee or tea, was permitted between midnight and 18.00 hours daily, and no smoking was allowed for 24 h before, or during, the study. The subjects were asked not to eat any food between leaving the unit each day and returning the next morning. Water, or a small amount of weak tea, coffee or cocoa, made with skimmed, or semi-skimmed milk, was permitted between 18.00 hours and midnight.

An intravenous indwelling cannula was inserted into an antecubital vein between 07.30 and 08.00 hours daily for the purpose of blood sampling. Blood samples were drawn without stasis in the fasting state 2 min before breakfast and at 30 min, 1, 2 and 4 h subsequent to starting breakfast and at 2 min before commencing lunch and 30 min, 1, 2 and 4 h subsequent to the start of lunch. On each occasion subjects were seated for 5 min before and during phlebotomy, the cannulas were flushed with heparinized saline and the first sample discarded.

Experiment 2

The purpose was to compare the effects of the consumption of two fibre supplements taken three times daily before meals for 3 weeks on fasting and PP responses of plasma lipids, glucose and insulin. Subjects were instructed to take the supplements following the procedure described below for day 22. The supplements consisted of 8·33 g of a powdered and refined fibre extract of the root of sugar beet, 0·4 g natural orange flavour and 0·6 g citric acid, providing 6 g dietary fibre (treatment A), and 2 g α-cellulose (BP grade), 0·4 g orange flavour and 0·6 g citric acid (treatment B, control, minimum fibre response).

There were eight subjects of each sex averaging 42·2 (SD 11·6) years (range 20 to 60 years). They were blocked in pairs with similar body weight within sex and the treatments were allocated at random within the blocks of a randomized block design. The mean BMI was 25·4 (SD 3·81) kg/m^2, and mean fasting plasma values were: total cholesterol 5·9 (SD 0·86) mmol/l, TAG 1·31 (SD 0·276) mmol/l, glucose 5·1 (SD 0·69) mmol/l, and insulin 6·9 (SD 2·22) μU/ml. On days 1 and 22, glucose tolerance tests were performed following a 12 h fast. No medication, food, drink or tobacco could be taken, other than that provided as part of the treatment, from 12 h before, or during, the tests. In sequence at 1 min intervals from 09.00 hours, subjects were given, over a period of 30 s, 330 ml tap water containing 60 g anhydrous glucose, 1·8 g citric acid and 1·2 g natural orange flavour. Venous blood was sampled as described for Expt 1, and sampling times were recorded relative to the start of consumption of the glucose solution. At 16 min before taking the glucose solution on day 1, in sequence at 1 min intervals, each individual consumed 150 ml tap water. On day 22 the treatment supplement was stirred into 150 ml water and it was consumed in 20 s. On days 2 to 21 the dietary habits were to remain the same as those prevailing during the previous month.

Blood analyses

Blood samples were treated with lithium heparin, apart from those used for glucose determination, which were mixed with potassium oxalate and sodium fluoride, and those for packed cell volume (PCV) measurement, which were treated with EDTA. PCV was measured in an automated cell counter (Medonic CA480; YSI Ltd, Farnborough, Hants). Plasma was separated by centrifugation at 4° and held at −20° in glass tubes. Plasma cholesterol was determined after hydrolysis of the ester and oxidation, using the method of Siedel *et al.* (1981). HDL-cholesterol was measured following the precipitation of lipoproteins other than HDL with phosphotungstic acid and Mg (Hitachi precipitant

543004). The cholesterol content of the resulting supernatant fraction was estimated by the method of Lopes-Virella *et al.* (1977). LDL-cholesterol was calculated using the formula of Friedwald *et al.* (1972) and plasma TAG was determined by enzymic hydrolysis and measurement of the glycerol produced. Plasma glucose was estimated by the method of Trinder (1969), using glucose oxidase (*EC* 1.1.3.4). Plasma immunoreactive insulin was determined using a kit manufactured by Radioassay Systems Laboratories Inc. (Los Angeles, CA, USA), cat. no. 134, Revision no. 4, and plasma albumin was measured colorimetrically using bromocresol green (Doumas *et al.* 1971) and Boehringer Mannheim (Lewes, East Sussex) reagents, cat. no. 79/342.

Diet analysis

Representative samples of each of the meals were taken and held at −20° before chemical analysis. Total lipids were determined by the modified method of Bligh and Dyer (Kirk & Sawyer, 1991), fatty acids by gas chromatography of methyl esters, crude protein by the Kjeldahl method, starch by the Ewer polarimetric method (Kirk & Sawyer, 1991), total sugars by the Luff and Schoorl method (Kirk & Sawyer, 1991), gross energy by adiabatic bomb calorimetry, dietary fibre was determined as non-starch polysaccharide (Englyst *et al.* 1982; Englyst & Cummings, 1984) and calcium by atomic absorption spectrophotometry. Starch, sugars and metabolizable energy of meals were also calculated from tables in Paul and Southgate (1978).

Statistical analyses

Expt 1 (4 d study). Blood was collected ten times on each of the 4 days. For each of the blood characteristics the analyses were carried out using the GENSTAT V, mark 4.03, with ANOVA and ANACOVA (analysis of covariance) routines, and the response curves for each variable, during the course of the day, described one of three forms, a, b or c. These were analysed as orthogonal comparisons, termed curve characteristics, three of which were common to the three forms. Six, seven and eight additional linear and quadratic characteristics were analysed for curves respectively of types a, b and c. The complete set of orthogonal Latin squares used allowed account to be taken of any carry-over effects from day to day within subject (Jones & Kenward, 1989). There was no evidence of such first- or second-order carry-over effects for any curve characteristic of any blood variable. The responses at times 2–10 were adjusted by covariance to the mean of time 1 for each blood variable. It was considered that treatment differences might be explained by haemoconcentration and therefore a covariance adjustment of response on plasma albumin was made. The insulin:glucose ratio was calculated for each observation of these variables and the ratio was analysed. For this a SAS PROC GLM program was used for the comparison, morning *v.* afternoon, as measured by area under the curve (AUC) and by peak height. A PROC REG program was used to compare morning *v.* afternoon, as measured by slope of increase of the ratio to the peak.

Expt 2 (3-week study). Covariance adjustment of the results for fasting values was made. Adjustment of responses for day 1 fasting values of the same variable had the purpose of taking account of baseline measurements. The simultaneous adjustment for both days 1 and 22 of that variable had the purpose of determining whether there was any additional effect of treatment to that on the day 22 fasting values. The results of Expt 1 had indicated that haemoconcentration may be affecting responses; therefore concurrent PCV values were also used as covariates.

Ethical considerations

The volunteers were selected for their plasma lipid and body-weight characteristics from the panel on dietary studies of Clinical Science Research International, Hinchingbrooke Hospital. The protocols prepared for each study were approved by the Huntingdon Area Health Authority Medical Ethics Committee and the volunteers gave their informed consent to the studies.

RESULTS

Experiment 1

All subjects consumed their meals. Mean eating times for breakfast and lunch were 16·2 (SED 2·18) min and 22·1 (SED 2·14) min respectively. There were no significant differences amongst the responses to the three fibre treatments, and after establishing that there were no first- or second-order carry-over effects between days the results for fibre treatments were meaned. The PP plasma concentrations of all response measurements, apart from TAG, increased rapidly for 30 min from the start of eating (Figs 1–5; $P < 0·01$). Subsequent to that the plasma concentrations of total cholesterol and its fractions declined. The post-breakfast concentrations of total and LDL-cholesterol for the controls were lower than the values for the mean of the fibre treatments, so that from the pre-breakfast fasting value the decrease to the mean of the PP morning, or to the mean of all the post-breakfast values, was greater for the control treatment (treatment difference 0·13 (SED 0·046) mmol/l in the morning and 0·13 (SED 0·040) mmol/l all day for total cholesterol and 0·13 (SED 0·044) mmol/l and 0·16 (SED 0·040) mmol/l respectively, for LDL-cholesterol, $P < 0·01$; Figs 1a, b). No significant difference between treated and control groups was found in plasma HDL-cholesterol or TAG concentration (for the comparison, pre-breakfast *v.* all post-breakfast values, differences were 0·007 (SED 0·0112) mmol/l for HDL-cholesterol and 0·004 (SED 0·0563) mmol/l for TAG; Fig. 2). HDL-cholesterol concentration declined from the pre-breakfast value (excluding the 30 min value) continuously to 2 h after the commencement of lunch ($P < 0·001$). The concentration of TAG rose continuously from 30 min following the start of breakfast until 1 h after lunch and it then declined. The mean within-subject PP changes in TAG and HDL-cholesterol concentrations were negatively correlated (r −0·926, $P < 0·001$, df 8), although the relationship for the fasting values of these two characteristics was not significant (r −0·464, not significant). Fasting TAG and maximum PP TAG concentrations were also correlated (r 0·697, $P < 0·05$).

The rise in plasma glucose concentration for 30 min from the commencement of breakfast was greater for those given the fibre treatments than for the controls (difference 0·37 (SED 0·163) mmol/l, $P < 0·05$; Fig. 3a), but after lunch there was no significant difference. The increase in insulin concentration after lunch was less for the fibre treatments than for the controls (difference 5·0 (SED 2·37) μU/ml, $P < 0·05$). The difference after breakfast was not significant (Fig. 3b). The peak insulin response to lunch was lower than that to breakfast ($P < 0·0405$), despite the lunch providing considerably more carbohydrate and protein than was provided by the breakfast (Table 2). The difference between control and test treatments for AUC of insulin : glucose ratio was not significant. Over the first three sampling times from the start of eating the linear increase in ratios to 60 min was greater for breakfast than for lunch ($P < 0·0001$, difference 0·056 (SED 0·0120) U/mol per min), and the peak height for breakfast was greater than that for lunch ($P < 0·0001$, respectively 12·8 and 9·7 (SED 0·35) U/mol, Fig. 4).

Plasma albumin concentration rose steeply from the fasting value for 30 min from the start of breakfast ($P < 0·001$) and lunch ($P < 0·001$) and the change postprandially from the fasting level was different for control and fibre treatments during the morning (difference 0·71 (SED 0·140) g /l, $P < 0·001$) and throughout the day (difference 0·56 (SED

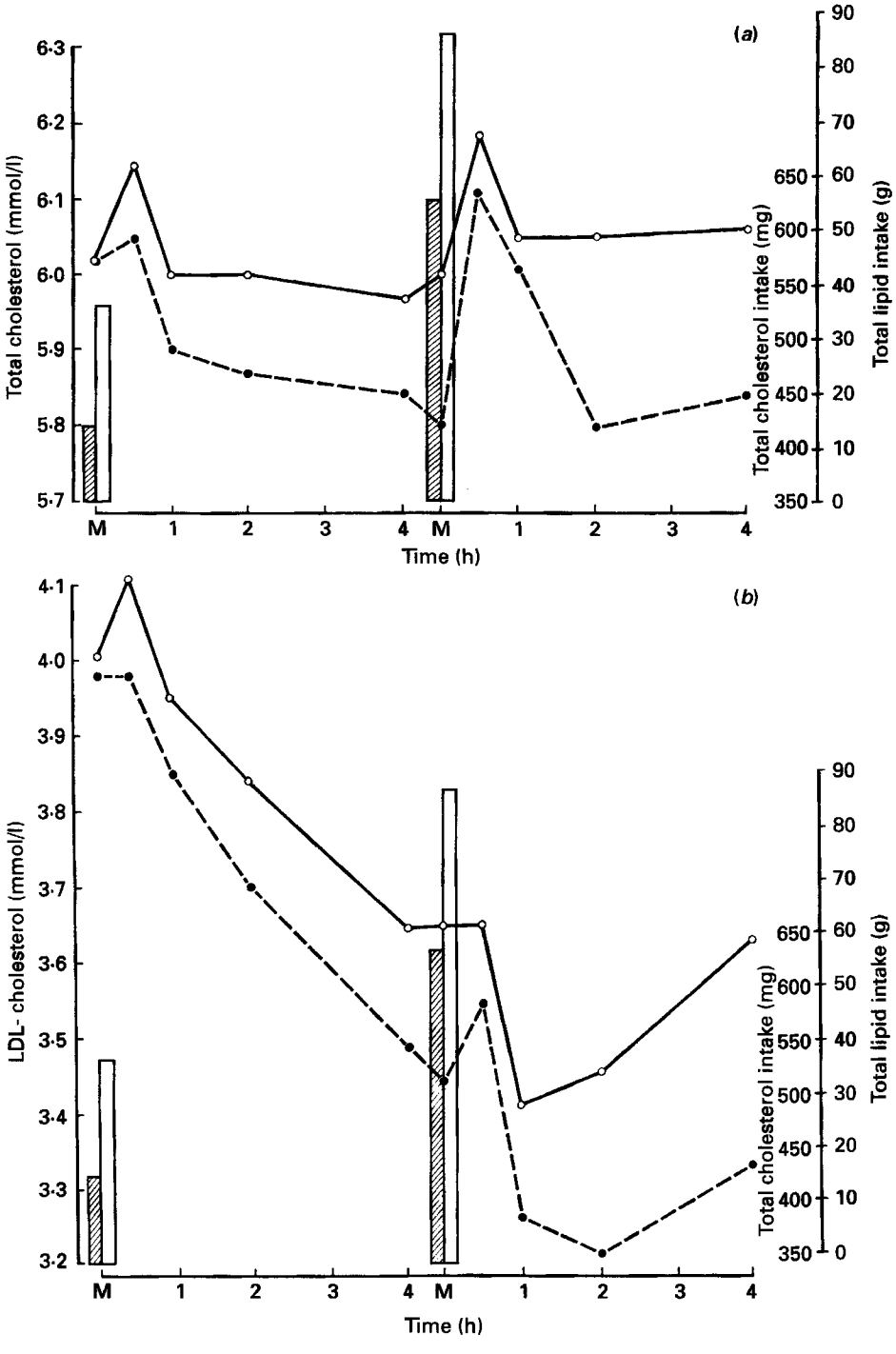

Fig. 1. Expt 1. Plasma total cholesterol (a) and LDL-cholesterol (b) concentrations (mmol/l) in subjects treated with psyllium with or without citrus pectin (○–○, mean of three treatments) and controls (●---●) following breakfast and lunch meals. Blood samples were collected at 0, 0·5, 1·0, 2·0 and 4·0 h following the commencement of each meal (M) on each of four days. Values are means for twelve subjects. Vertical bars represent the weight of cholesterol (▩) and total lipid (□) in each meal. For details of diets and procedures, see Tables 1 and 2 and pp. 734–737.

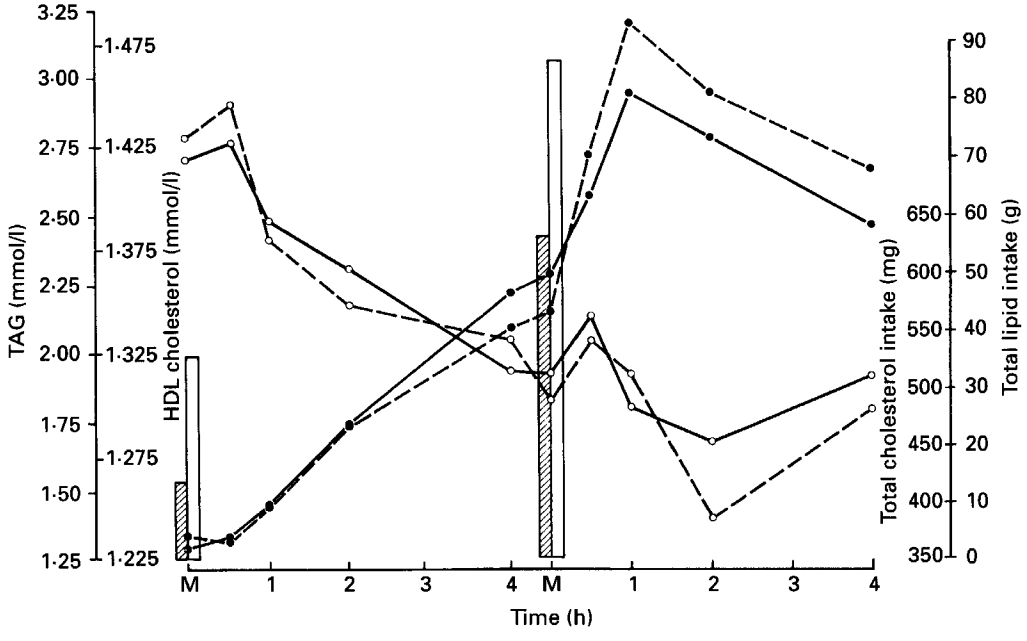

Fig. 2. Expt 1. Plasma HDL-cholesterol (○) and triacylglycerol (TAG; ●) concentrations (mmol/l) in subjects treated with psyllium with or without citrus pectin (—, mean of three treatments) and controls (---) following breakfast and lunch meals. Blood samples were collected at 0, 0·5, 1·0, 2·0 and 4·0 h following the commencement of each meal (M) on each of four days. Values are means for twelve subjects. Vertical bars represent the weight of cholesterol (▨) and total lipid (□) in each meal. For details of diets and procedures, see Tables 1 and 2 and pp. 734–737.

0·103) g/l, $P < 0·001$). The linear decrease from 30 min after lunch was less for the fibre than for the control treatment (difference 0·097 (SED 0·0343) g/l, $P < 0·05$; Fig. 5). Plasma concentrations of total cholesterol and albumin were correlated (r 0·869, $P < 0·01$) within subject. The covariance on plasma albumin for plasma insulin, or glucose, was not significant, whereas that for total, LDL- and HDL-cholesterol was, and the treatment differences in the total and LDL fractions were removed following adjustment.

Experiment 2

On day 22 there were lower fasting plasma total ($P = 0·001$) and LDL ($P = 0·011$)-cholesterol values for sugar-beet fibre (A) than for cellulose (B), but no significant treatment difference was found in fasting plasma HDL-cholesterol. The treatment A fasting TAG mean was not significantly lower than B. The mean difference was 0·16 mmol/l (12·5%; Table 3). Treatment had no effect on fasting plasma glucose or insulin concentrations on day 22.

On day 22 the mean plasma total and LDL-cholesterol values at 128 min and 256 min postprandially for beet fibre (A) were lower than those for cellulose (B) with probabilities respectively of 0·011 and 0·012 for total cholesterol and respectively of 0·010 and 0·040 for LDL-cholesterol, when day 1 fasting values only were used as covariates. No treatment difference for either response occurred when covariance for fasting values on both days was applied (Table 3). There was no significant PP change in plasma HDL-cholesterol level for either treatment on day 1, but on day 22 at 128 min the mean value for beet fibre (A) was

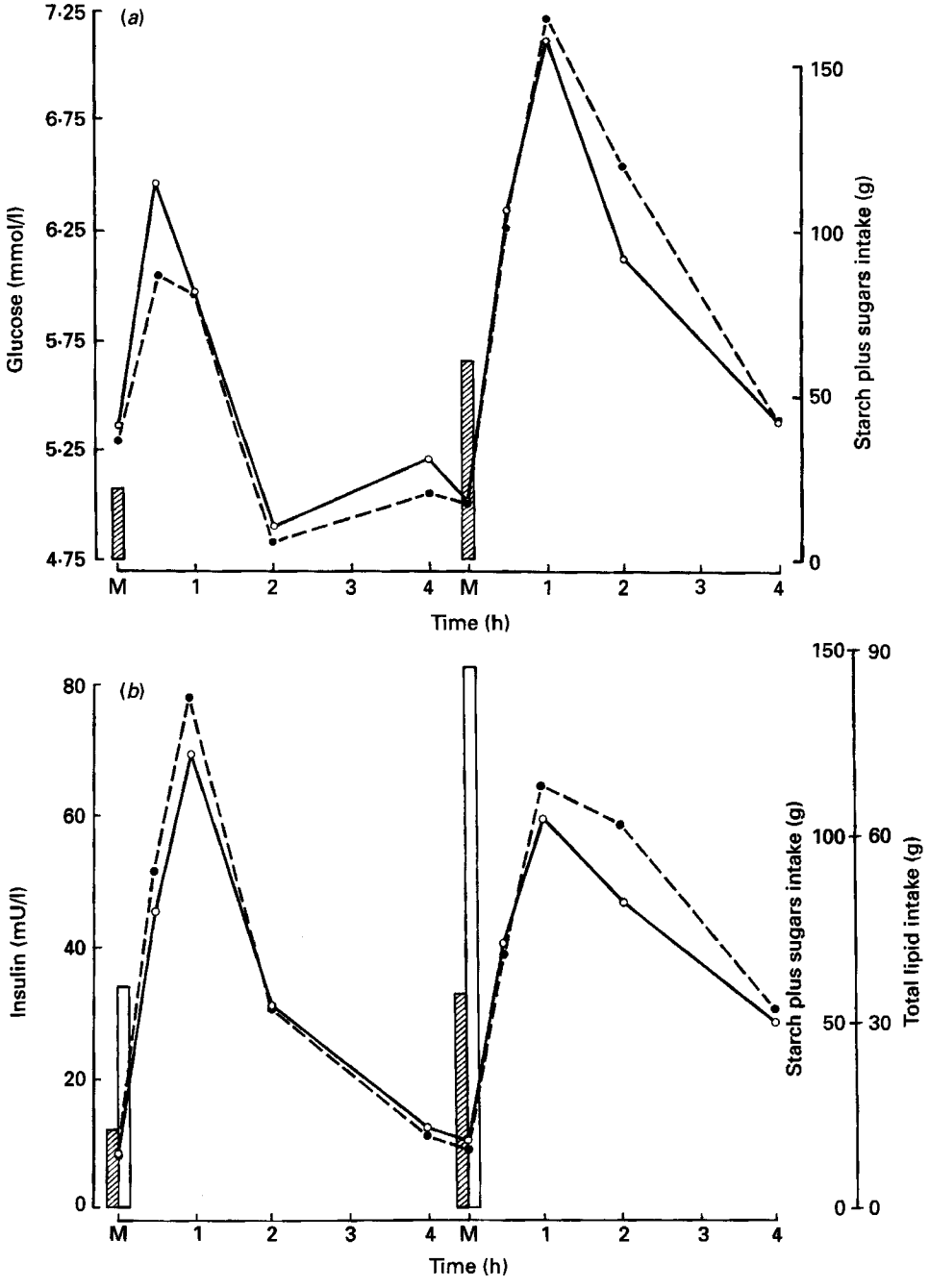

Fig. 3. Expt 1. Plasma glucose concentration (mmol/l) (a) and plasma insulin concentration (mU/l) (b) in subjects treated with psyllium with or without citrus pectin (○–○, means of three treatments) and controls (●–●) following breakfast and lunch meals. Blood samples were collected at 0, 0·5, 1·0, 2·0 and 4·0 h following the commencement of each meal (M) on each of four days. Values are means for twelve subjects. Vertical bars represent the weight of starch + sugars (▨) and total lipid (□) in each meal. For details of diets and procedures, see Tables 1 and 2 and pp. 734–737.

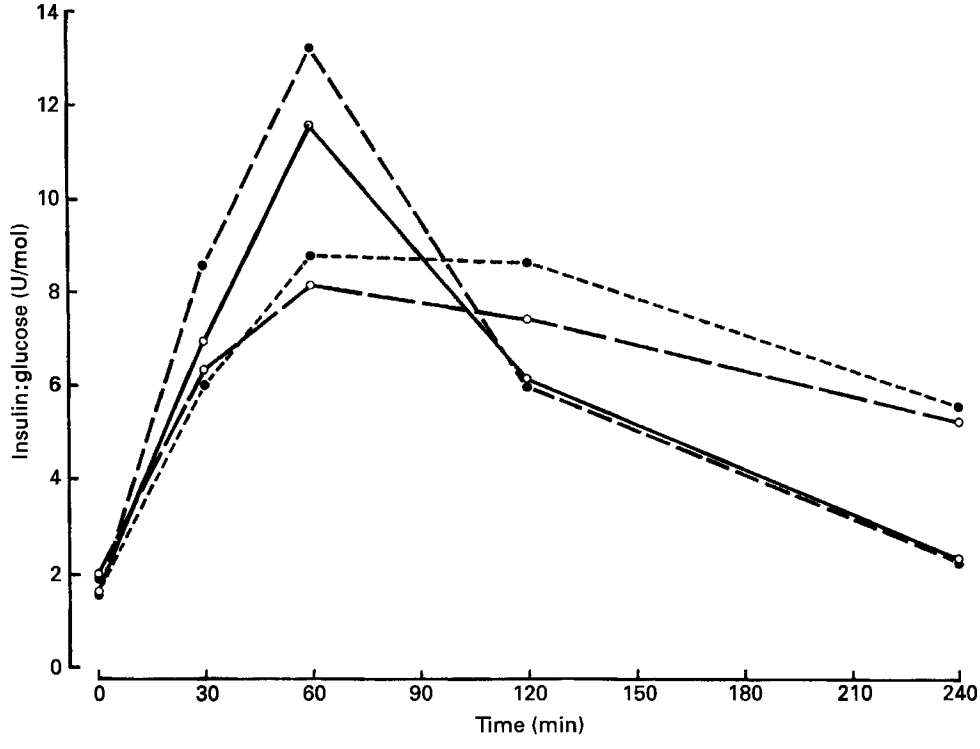

Fig. 4. Expt 1. Plasma insulin: glucose ratio (U/mol) in subjects treated with psyllium with or without citrus pectin (○, mean of three treatments) and controls (●) following breakfast (○–○ and ●–●) and lunch (○——○ and ●---●) meals. Blood samples were collected at 0, 0·5, 1·0, 2·0 and 4·0 h following the commencement of each meal on each of four days. Values are means for twelve subjects. For details of diets and procedures, see Tables 1 and 2 and pp. 734–737.

0·08 mmol/l higher than that for cellulose (B), using both days 1 and 22 fasting values as covariates ($P = 0·011$). The values for A also rose from fasting to 128 min on day 22 ($P < 0·05$). Covariance adjustment of PP TAG values for both days 1 and 22 fasting values removed most of the apparent difference between treatments at 128 min or 256 min. The overall mean TAG values were approximately the same at fasting and 128 min, but had risen sharply by 256 min (Table 3). The multiple covariance adjustment indicates that only in the case of HDL-cholesterol was there a measurable independent effect of treatment on PP response that could not be accounted for by the effect of treatment on fasting response.

Treatment influenced the response curves of both glucose and insulin on day 22 (Table 4). Beet fibre (A) lowered the plasma glucose response at 16 min following adjustment for the fasting value on day 22 ($P = 0·025$) and the further inclusion of concurrent PCV maintained this treatment difference ($P = 0·019$). The decrease in AUC for glucose from day 1 to day 22 was 6·9% for beet fibre relative to the cellulose control ($P = 0·066$, Table 5). Beet fibre reduced the insulin response from day 1 to day 22 at 32 min and 256 min ($P = 0·042$ and $P = 0·040$), but a difference between treatments in the change in AUC of 9·6% was not significant.

The PCV and plasma albumin values are given in Fig. 6 with the mean albumin value for breakfast in Expt 1. There was a slight rise in PCV during the first hour after glucose consumption on days 1 and 22, but the time effect was not significant for Expt 2. The

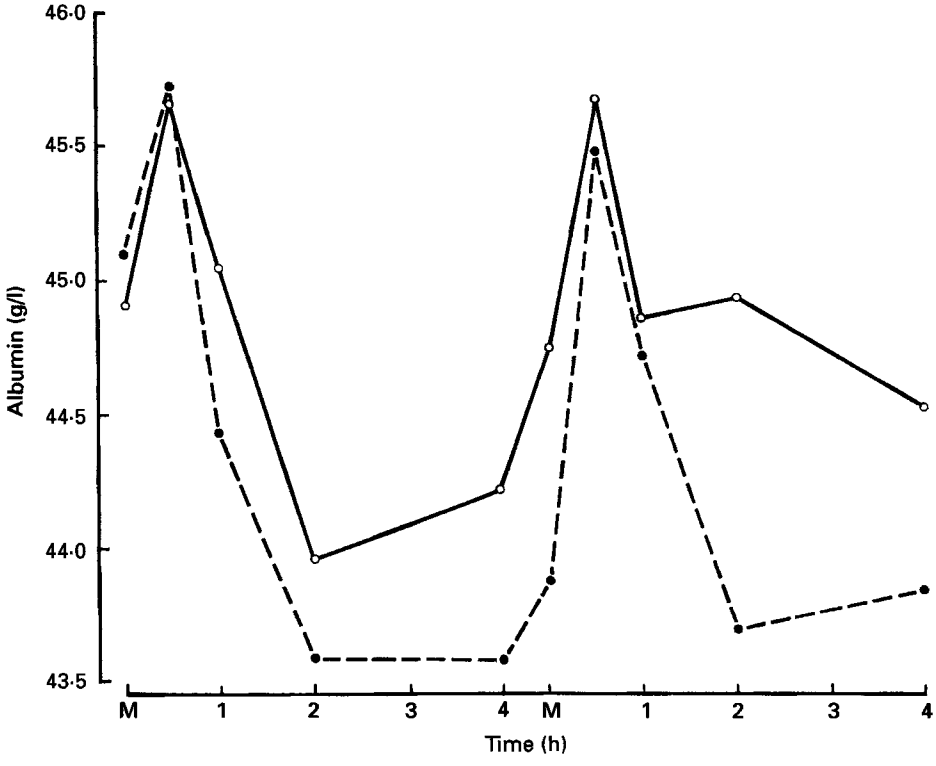

Fig. 5. Expt 1. Plasma albumin concentration (g/l) in subjects treated with psyllium with or without citrus pectin (○–○, mean of three treatments) and controls (●---●) following breakfast and lunch meals. Blood samples were collected at 0, 0·5, 1·0, 2·0 and 4·0 h following commencement of each meal (M) on each of four days. Values are means for twelve subjects. For details of diets and procedures, see Tables 1 and 2 and pp. 734–737.

changes in albumin concentration with time were significant on day 1 ($P = 0{\cdot}014$), but not so on day 22 ($P = 0{\cdot}118$), and were in accord with the PCV changes. The values rose steeply to 16 min and had returned to the fasting values by 64 min in both treatment groups. Covariance on PCV in Expt 2 was significant for plasma concentrations of total, HDL- and LDL-cholesterol and glucose. The only resulting significant change in the treatment comparisons, of values adjusted for fasting effects, was that of glucose referred to above.

DISCUSSION

Plasma glucose and insulin responses to fibre intake

It is generally agreed that viscous dietary fibre influences glucose metabolism by retarding the rate of glucose absorption (Johnson, 1990; Mazur *et al.* 1990; Roberts *et al.* 1990), so that effects on fasting plasma levels of insulin and glucose would not generally be observed in normal subjects, unless large doses of fibre are given (Landin *et al.* 1992). The absence of effects of beet fibre on fasting blood in Expt 2 are in accord with this. A reduced rate of glucose absorption influences the glycaemic response and hence the insulin response. The consumption of 6 g beet fibre per meal for 3 weeks was associated with effects at specific points on the PP response curves of both insulin and glucose. Decreases in AUC of 9·6% (not significant) and 6·9% ($P = 0{\cdot}066$) for insulin and glucose respectively also occurred

Table 3. *Expt 2. Plasma lipid responses on day 22 (mmol/l) with covariance adjustment for fasting values on day 1 (a), unadjusted values (b) and values adjusted for fasting values on both days 1 and 22 (c) for subjects receiving sugar-beet fibre (A) and controls (B)**

(Mean values and standard errors of differences between means, SED)

Time after meal (min)...		0		128		256		128	256
		a	b	a	b	a	b	c	c
Total cholesterol	A	5·40	5·36	5·47	5·43	5·47	5·33	5·72	5·69
	B	5·90	5·94	5·90	5·93	6·02	6·16	5·64	5·80
	SED	0·083	0·455	0·119	0·400	0·141	0·328	0·237	0·317
HDL-cholesterol	A	1·30	1·27	1·48	1·45	1·33	1·27	1·51	1·37
	B	1·43	1·46	1·46	1·49	1·47	1·53	1·43	1·43
	SED	0·103	0·164	0·051	0·136	0·083	0·120	0·020	0·082
LDL-cholesterol	A	3·51	3·60	3·44	3·52	3·51	3·50	3·63	3·71
	B	3·87	3·79	3·91	3·83	3·86	3·86	3·72	3·65
	SED	0·100	0·354	0·124	0·386	0·126	0·365	0·137	0·201
TAG	A	1·12	1·02	1·06	0·93	1·30	1·16	1·15	1·33
	B	1·28	1·38	1·21	1·35	1·50	1·64	1·12	1·46
	SED	0·223	0·214	0·267	0·267	0·205	0·247	0·117	0·114

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, triacylglycerol.
* For details of diets and procedures, see pp. 736–737.

Table 4. *Expt 2. Fasting and postprandial plasma glucose and insulin concentrations in subjects treated with sugar-beet fibre (A) and controls (B) at various times after a bolus glucose dose (day 1) or a fibre supplement and a bolus glucose dose (day 22)**

(Mean values and standard errors of the difference between means, SED)

Time (min)...		0	16	32	64	128	256
Glucose (mmol/l)							
Day 1	A	5·17	6·52	7·60	7·48	5·28	4·42
	B	5·03	6·88	7·41	6·28	4·50	4·31
	SED	0·344	0·383	0·434	1·014	0·433	0·229
Day 22†	A	4·89	6·20	6·91	6·72	4·51	4·27
	B	4·82	6·68	7·53	6·01	4·20	4·49
	SED	0·092	0·185	0·351	0·781	0·540	0·238
Insulin (μU/ml)							
Day 1	A	6·8	26·4	41·1	48·3	25·4	5·7
	B	7·1	28·9	45·2	56·5	17·8	5·6
	SED	1·11	7·41	12·40	14·95	4·98	0·73
Day 22†	A	7·9	36·0	46·3	48·2	21·5	6·8
	B	9·1	46·1	63·9	54·7	14·9	7·7
	SED	1·24	10·99	11·61	13·29	6·66	0·856

* For details of diets and procedures, see pp. 736–737.
† Values adjusted by covariance for fasting values on day 1.

Table 5. *Expt 2. Areas under the curve for plasma glucose (mmol.min/l) and insulin (μU.min/ml) in subjects treated with sugar-beet fibre (A) and controls (B) on days 1 (no fibre supplements) and 22 (with fibre supplements) at 0–265 min after receiving a bolus glucose dose**

(Mean values for eight subjects, and standard errors of the differences between treatments (SED))

	Glucose (mmol.min/ml)			Insulin (μU.min/ml)		
Day...	1	22	(22−1)	1	22	(22−1)
Group A	1477	1340	−137	6579	6508	−71
Group B	1338	1298	−40	6382	6932	+550
SED	87·1	84·6	44·5	1207·2	1244·5	718·3
P	0·15	0·63	0·066	0·87	0·74	0·42

* For details of diets and procedures, see pp. 736–737.

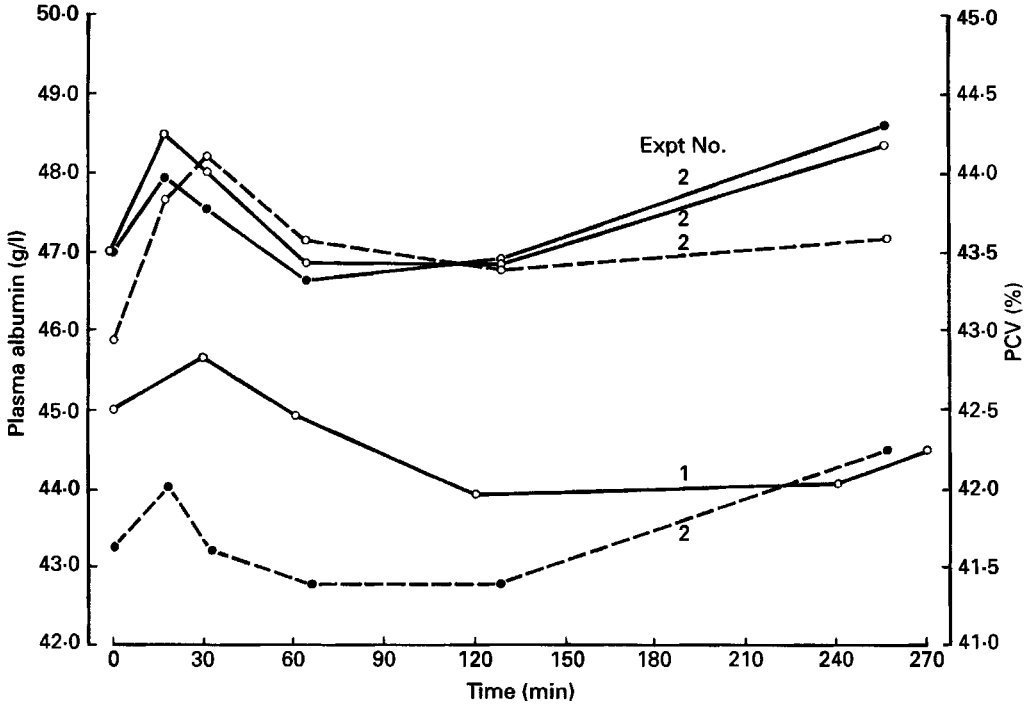

Fig. 6. Expts 1 and 2. Plasma albumin concentrations (g/l; —) and blood packed cell volume (PCV, %; ---) at various times up to 256 min from the start of breakfast in Expt 1 or after giving a glucose solution in Expt 2. Measurements were taken on day 1 (○) and day 22 (●). Values are means of all subjects. For details of procedures, see pp. 734–737.

(Table 5). The inherent variability of these responses is generally large, so that repeatable effects do not occur in healthy subjects unless large doses of viscous fibre are given (Braaten *et al.* 1991). In the acute Expt 1 no glycaemic effects occurred for 2·2 g water-soluble fibre and it may indicate that either metabolic experience of effective fibre sources promotes PP glycaemic effects, or that quantities greater than 2–3 g fibre per meal are required.

Plasma cholesterol and fibre intake

Can the effect of dietary fibre on plasma cholesterol be measured postprandially? In several studies no reduction of plasma fasting cholesterol was found with chronic dietary fibre supplementation (Hillman *et al.* 1985; Leadbetter *et al.* 1991; Tredger *et al.* 1991). In several others soluble or insoluble fibre fractions yielded fasting cholesterol reductions in rats (Horigome *et al.* 1992) and in human subjects (Anderson *et al.* 1991, 1992; Cara *et al.* 1992*b*; Landin *et al.* 1992; Zhang *et al.* 1992). As little as 2–3 g dietary fibre/d, with some fat, as wheat germ, has been effective (Cara *et al.* 1992*a*), but soluble, or partly soluble, fibre is much more effective than insoluble (Flynn *et al.* 1991; Kashtan *et al.* 1992). The reported daily amounts of supplemental total dietary fibre have tended to be greater than we used, yet the amounts of soluble fibre have been in the same range as those we provided. In consequence the absence of a fibre-induced lowering effect on PP plasma cholesterol in Expt 1 is not accounted for by these factors, and was considered to result from consumption of the fibre as a tablet before the meal (Fairchild *et al.* 1990). On the other hand, plasma fasting cholesterol (Uusitupa *et al.* 1989; Landin *et al.* 1992) and PP glucose (Fairchild *et al.* 1993) have been decreased when guar gum was given before meals, in accord with the effectiveness of beet fibre in Expt 2. Thus the absence of an independent effect of fibre on PP plasma total or LDL-cholesterol concentration in Expts 1 and 2 indicates that fibre efficacy may not be measured by PP changes in plasma concentration. In support of this McCall *et al.* (1992*a*, *b*) demonstrated that psyllium husk probably lowered plasma cholesterol in monkeys by decreasing LDL synthesis, and not by affecting cholesterol absorption.

Plasma HDL-cholesterol and TAG concentration responses to fibre

The plasma concentration of fasting HDL-cholesterol is generally not increased, nor that of TAG markedly decreased, by the chronic intake of fibre supplements at normal rates (Hillman *et al.* 1985; Shutler *et al.* 1989; Anderson *et al.* 1991; Leadbetter *et al.* 1991; Tredger *et al.* 1991; Kashtan *et al.* 1992; Zhang *et al.* 1992). Accordingly a dose of 6 g dietary beet fibre per meal in Expt 2 did not significantly lower fasting concentrations of TAG (treatment difference 12·5%), but TAG was lowered significantly, although marginally, in another chronic study (D. L. Frape and A. M. Jones, unpublished results). Cara *et al.* (1992*b*) found that small amounts of raw and partially defatted wheat germ chronically decreased TAG without influencing fasting HDL-cholesterol in hypercholesterolaemic, hypertriacylglycerolaemic patients and Landin *et al.* (1992) reported that large daily doses of guar gum lowered plasma TAG. Acute effects of fibre on TAG concentration seem even less likely, as found in Expt 1 and by Lovegrove *et al.* (1993).

PP changes in plasma TAG and HDL-cholesterol concentrations were highly correlated within subject in Expt 1 (r −0·926, $P < 0·001$) and between subjects in chronic studies ($P < 0·001$, D. L. Frape and A. M. Jones, unpublished results). Similar relationships have been reported in both insulin-dependent diabetics (Rillaerts *et al.* 1989), and healthy (Kashyap *et al.* 1983) and obese (Akanji *et al.* 1992) subjects. Elevated plasma TAG seems to be the underlying cause of reduced concentrations of plasma HDL_2 (Patsch *et al.* 1983) and HDL-cholesterol, depressed in subjects with insulin resistance (Dean *et al.* 1991). The insulin sensitivity of adipocytes affects both glucose metabolism and lipoprotein lipase (*EC* 3.1.1.34, LPL) secretion (Fried *et al.* 1990). Adipose-tissue LPL activity modulates plasma concentrations of HDL-cholesterol (Eisenberg *et al.* 1984) and probably of HDL_2-cholesterol (Taskinen & Nikkilä, 1981) by influencing PP plasma TAG concentration (Tall, 1986*a*). Elevated plasma TAG activates cholesterol-ester transfer protein (CETP), which transfers cholesterol ester (CE) from HDL to less dense lipoproteins after a fat meal (Tall,

1986*a*, *b*; Cohn *et al.* 1988). In Expts 1 and 2 the mean HDL-cholesterol values were respectively 0·936 and 1·069 mmol/l (males) and 1·552 and 1·690 mmol/l (females) (SED 0·1677 and 0·1640 mmol/l). The relationship between plasma concentrations of HDL-cholesterol and TAG was significantly closer for males than for females in Expt 1 and significant for males only in Expt 2 (r −0·761, $P < 0·05$ on day 1 and r −0·846, $P < 0·01$ on day 22). This may indicate that CETP was more active in the males in which PP plasma TAG rose 22% more than it did in the females of Expt 1, in accord with other observations (Cohn *et al.* 1988), causing greater atherogenic potential both in males (Kashyap *et al.* 1983; Tall, 1986*a*) and with increasing age (Cohn *et al.* 1988). In CETP deficiency there are no clinical signs of IHD (Schmitz & Williamson, 1991). Our mean peak plasma TAG values in Expt 1 were in excess of those recorded by Groot & Scheek (1984) in younger individuals, and the direction and magnitude of the PP change in plasma HDL-cholesterol concentration would seem to depend on the degree to which CETP is activated to counter the transfer of cholesterol to HDL from chylomicrons and very-low-density lipoproteins (VLDL). Fielding *et al.* (1989) found that cholesterol is transferred postprandially to HDL from VLDL and LDL in chow-adapted baboons, but the reverse occurred in those adapted to fat. This mechanism should account for: (1) the PP rise and treatment difference in HDL-cholesterol with chronic fibre supplementation in Expt 2 following a glucose dose ($P = 0·011$) and after a low-fat breakfast (D. L. Frape & A. M. Jones, unpublished results), and (2) the acute decline following fatty meals in Expt 1 ($P < 0·001$). Accordingly no acute effect of fibre on PP HDL-cholesterol would be expected, but a chronic effect could depend on soluble fibre increasing insulin sensitivity (as indicated by the PP insulin response to beet fibre) of adipocytes to stimulate LPL secretion, which would accelerate chylomicron clearance so that CETP is not activated.

Diurnal patterns of lipid metabolism

Diurnal patterns of lipid metabolism have been reported. The maximum fractional synthetic rate of cholesterol may occur at about 06.00 hours (Jones & Schoeller, 1990). Mirani-Oostdijk *et al.* (1983) recorded a decrease in plasma HDL-cholesterol from 09.00 to 13.00 hours. This decrease agrees with our observations in Expt 1 after a high-fat breakfast, but not with those after a very-low-fat breakfast (D. L. Frape & A. M. Jones, unpublished results), or after an oral glucose dose in the earning morning (Expt 2). The plasma TAG response in Expt 1 indicated that the peak was reached between 30 min and 2 h after lunch, but not even in 4 h after breakfast, despite a much lower fat content to the breakfast. A wave-like daily pattern in plasma TAG concentration is associated with highest values for adipose tissue LPL activity at 16.30 and 21.30 hours, and low values at 08.30 hours (Mirani-Oostdijk *et al.* 1983). Low values at this time are consistent with a depressed adipose-tissue LPL response to insulin following a short fast (Fried *et al.* 1990). As this response modulates CETP function it may explain, (1) the short duration of the TAG and HDL-cholesterol responses to lunch relative to those at breakfast in Expt 1, (2) the extended duration of the TAG response to a meal containing 47 g fat at 09.00 hours (D. L. Frape & A. M. Jones, unpublished results), compared with the response to a meal containing 87 g fat in Expt 1 given at 13.00 hours, and (3) the maintenance of HDL-cholesterol concentration following chronic fibre supplementation and a glucose dose at 09.00 hours in Expt 2.

Observations were made in Expt 1 of lower peak plasma insulin and insulin:glucose ratio responses to lunch than to breakfast (Figs 3b, 4), despite the provision at lunch of much larger amounts of insulinotropic protein-bound amino acids (Gulliford *et al.* 1989; Nordt *et al.* 1991) and of glucose-yielding carbohydrates, to which the response is proportional (Macdonald *et al.* 1978; Rasmussen *et al.* 1992). Dietary lipid is unlikely to have influenced

the comparison of insulin responses at breakfast and lunch, as fat-stimulated incretin secretion does not influence insulin secretion (Morgan, 1992), and lipid incorporation into meals has no acute effect on PP plasma insulin concentration (Collier & O'Dea, 1983; Gulliford *et al.* 1989; Nordt *et al.* 1991). Blackard *et al.* (1989) suggested that the difference in response to breakfast and lunch could be eliminated by providing rapidly absorbed carbohydrate for breakfast and that a putative 'dawn phenomenon' of insulin resistance (Feo *et al.* 1988) is more likely owing to a lack of residual insulin. Plasma insulin and glucose concentrations before breakfast were similar to those before lunch in Expt 1. It is possible that diurnal changes in HDL metabolism result from insulin resistance and reduced adipose-tissue LPL activity in the early morning.

Plasma albumin and PCV as indicators of haemoconcentration

In Expt 1, following both breakfast and lunch, an immediate rise occurred in plasma concentrations of total, HDL- and LDL-cholesterol and albumin ($P < 0{\cdot}01$). The values for total, HDL- and LDL-cholesterol reached maxima and had declined within 1 h from the start of eating. Values for plasma albumin were higher in samples from the fibre treatments than for the controls between breakfast and lunch ($P < 0{\cdot}001$), and with a less steep decline after lunch, the 2 h and 4 h albumin values were also higher for fibre ($P < 0{\cdot}05$; Fig. 5). The concentrations of albumin and total cholesterol were correlated (r 0·87, $P < 0{\cdot}001$) and covariance adjustment for albumin removed the treatment effect, suggesting that the higher total cholesterol for the fibre treatment was a haemoconcentration effect. The time means for albumin in Expt 2 were significantly different on day 1 ($P = 0{\cdot}014$; Fig. 6). Covariance adjustment for PCV in Expt 2 increased the significance of the beet-fibre effect on glucose tolerance. If soluble fibre sources cause a relative increase in PP haemoconcentration, ignoring this in measurement of glycaemic response may underestimate fibre's power to improve glucose tolerance. Water consumed with meals does not influence the size of this response to a meal (Gregersen *et al.* 1990), and water consumption alone causes a rise in plasma protein concentration of horses (Meyer *et al.* 1990). The act of drinking, or eating and particularly the consumption of water adsorbant fibre, would seem to provoke an initial net fluid shift out of the plasma into the gastrointestinal tract lumen. This fluid is probably a component of digestive secretions. The maximum effect was at approximately 30 min from the start of a meal in agreement with observations in an unreported experiment of one of the authors (DLF).

CONCLUSIONS

Low doses of psyllium gum have no acute effect on carbohydrate and lipid markers of IHD risk during the postprandial period in volunteers with mildly increased risk.

Sugar-beet dietary fibre, 6 g, taken three times daily as a powder in water before meals for 3 weeks lowers the concentrations of total and LDL-cholesterol in the blood plasma following a 12 h fast on the 22nd day. In a subsequent glucose tolerance test the beet fibre, taken before the glucose, improves tolerance. A lower plasma concentration of total and LDL-cholesterol during glucose assimilation in subjects receiving the chronic fibre treatment is accounted for by fasting differences in plasma concentration of these metabolites.

Change in haemoconcentration measured by plasma albumin, or PCV, was found to influence the PP measurements in both experiments. If no account is taken of this effect the improvement in glucose tolerance, caused by dietary fibre, could be underestimated.

The high-fat breakfast caused a continuous decline in plasma HDL-cholesterol concentration, associated with a considerable lipaemia, whereas the fatty lunch did not. The PP plasma insulin:glucose ratio reached a greater peak after breakfast than after

lunch. It is proposed that: (1) these differences are functionally related to a slower TAG clearance in the morning that in turn promotes transfer of cholesterol from HDL to lower density atherogenic lipoproteins, and that (2) dietary fibre can influence plasma HDL-cholesterol concentration by a chronic influence on insulin sensitivity of adipocytes.

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REFERENCES

Akanji, A. O., Nzegwu, A. A. & Agbedana, E. O.(1992). Some determinants of postprandial lipaemia in Nigerian diabetic and non-diabetic subjects. *British Journal of Nutrition* **68**, 153–162.

Anderson, J. W., Garrity, T. F., Wood, C. L., Whitis, S. E., Smith, B. M. & Oeltgen, P. R. (1992). Prospective, randomized, controlled comparison of the effects of low-fat and low-fat plus high-fiber diets on serum lipid concentrations. *American Journal of Clinical Nutrition* **56**, 887–894.

Anderson, J. W., Riddell-Lawrence, S., Floore, T. L., Dillon, D. W. & Oeltgen, P. R. (1991). Bakery products lower serum cholesterol concentrations in hypercholesterolemic men. *American Journal of Clinical Nutrition* **54**, 836–840.

Blackard, W. G., Barlascini, C. O., Clore, J. N. & Nestler, J. E. (1989). Morning insulin requirements. Critique of dawn and meal phenomena. *Diabetes* **3**, 273–277.

Braaten, J. T., Wood, P. J., Scott, F. W., Riedel, K. D., Poste, L. M. & Collins, M. W. (1991). Oat gum lowers glucose and insulin after an oral glucose load. *American Journal of Clinical Nutrition* **53**, 1425–1430.

Cara, L., Armand, M., Borel, P., Senft, M., Portugal, H., Pauli, A.-M., Lafont, H. & Lairon, D. (1992*a*). Long-term wheat germ intake beneficially affects plasma lipids and lipoproteins in hypercholesterolemic human subjects. *Journal of Nutrition* **122**, 317–326.

Cara, L., Dubois, C., Borel, P., Armand, M., Senft, M., Portugal, H., Pauli, A.-M., Bernard, P.-M. & Lairon, D. (1992*b*). Effects of oat bran, rice bran, wheat fiber, and wheat germ on postprandial lipemia in healthy adults. *American Journal of Clinical Nutrition* **55**, 81–88.

Cohn, J. S., McNamara, J. R., Cohn, S. D., Ordovas, J. M. & Schaefer, E. J. (1988). Postprandial plasma lipoprotein changes in human subjects of different ages. *Journal of Lipid Research* **29**, 469–479.

Collier, G. & O'Dea, K. (1983). The effect of coingestion of fat on the glucose, insulin, and gastric inhibitory polypeptide responses to carbohydrate and protein. *American Journal of Clinical Nutrition* **37**, 941–944.

Dean, J. D., Jones, C. J. H., Hutchinson, S. J., Peters, R. J. & Henderson, A. H. (1991). Hyperinsulinaemia and microvascular angina ('syndrome X'). *Lancet* **337**, 456–457.

Doumas, B., Watson, W. & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta* **31**, 87–96.

Eisenberg, S., Gavish, D., Oschry, Y., Fainaru, M. & Deckelbaum, J. (1984). Abnormalities in very low, low, and high density lipoproteins in hypertriglyceridemia. Reversal toward normal with Bezafibrate treatment. *Journal of Clinical Investigation* **74**, 470–482.

Englyst, H. N. & Cummings, J. H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas–liquid chromatography of constituent sugars as the alditol acetates. *Analyst* **109**, 937–942.

Englyst, H. N., Wiggins, H. S. & Cummings, J. H. (1982). Determination of the non-starch polysaccharides in plant foods by gas–liquid chromatography of constituent sugars as alditol acetates. *Analyst* **107**, 307–318.

Fairchild, R. M., Daniels, C. E. J., Ellis, P. R., Naqvi, S. H. M., Kwan, R. M. F. & Mir, M. A. (1990). Effect of two types of guar gum in solid and liquid foods on postprandial blood glucose, plasma insulin and C-peptide in healthy subjects. *Proceedings of the Nutrition Society* **49**, 54A.

Fairchild, R. M., Ellis, P. R., Kwan, R. M. F., Byrne, A. D. & Mir, M. A. (1993). The effect of two types of guar gum in solid and liquid foods on postprandial blood glucose, plasma insulin and C-peptide in obese subjects. *Proceedings of the Nutrition Society* **52**, 378A.

Feo, P. De, Perriello, G. & Bolli, G. B. (1988). Somogyi and dawn phenomena: mechanisms. *Diabetes/Metabolism Reviews* **4**, 31–49.

Fielding, P. E., Jackson, E. M. & Fielding, C. J. (1989). Chronic dietary fat and cholesterol inhibit the normal postprandial stimulation of plasma cholesterol metabolism. *Journal of Lipid Research* **30**, 1211–1217.

Flynn, M. A. T., Sugrue, D. D. & Gibney, M. J. (1991). The effect of rice and wheat bran supplements on plasma lipids in healthy adult male volunteers. *Proceedings of the Nutrition Society* **50**, 208A.

Fried, S. K., Velazquez, N. & Nobel, J. (1990). Nutrition-induced variations in responsiveness to insulin effects on lipoprotein lipase activity in isolated rat fat cells. *Journal of Nutrition* **120**, 1087–1095.

Friedewald, W. T., Levy, R. I. & Frederickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18**, 499.

Gregersen, S., Rasmussen, O., Winther, E. & Hermansen, K. (1990). Water volume and consumption time: influence on the glycemic and insulinemic responses in non-insulin-dependent diabetic subjects. *American Journal of Clinical Nutrition* **52**, 515–518.

Groot, P. H. E. & Scheek, L. M. (1984). Effects of fat ingestion on high density lipoprotein profiles in human sera. *Journal of Lipid Research* **25**, 684–692.

Gulliford, M. C., Bicknell, E. J. & Scarpello, J. H. (1989). Differential effect of protein and fat ingestion on blood glucose responses to high- and low-glycemic-index carbohydrates in non-insulin-dependent diabetic subjects. *American Journal of Clinical Nutrition* **50**, 773–777.

Hillman, L. C., Peters, S. G., Fisher, C. A. & Pomare, E. W. (1985). The effects of the fiber components pectin, cellulose and lignin on serum cholesterol levels. *American Journal of Clinical Nutrition* **42**, 207–213.

Horigome, T., Sakaguchi, E. & Kishimoto, C. (1992). Hypocholesterolaemic effect of banana (*Musa sapientum* L. var. Cavendishii) pulp in the rat fed on a cholesterol-containing diet. *British Journal of Nutrition* **68**, 231–244.

Johnson, I. T. (1990). Fibre sources for the food industry. *Proceedings of the Nutrition Society* **49**, 31–38.

Jones, B. & Kenward, M. G. (1989). *Design and Analysis of Cross-over Trials*, chapter 5. London: Chapman and Hall.

Jones, P. J. H. & Schoeller, D. A. (1990). Evidence for a diurnal periodicity in human cholesterol synthesis. *Journal of Lipid Research* **31**, 667–673.

Kashtan, H., Stern, H. S., Jenkins, D. J. A., Jenkins, A. L., Hay, K., Marcon, N., Minkin, S. & Bruce, W. R. (1992). Wheat-bran and oat-bran supplements' effects on blood lipids and lipoproteins. *American Journal of Clinical Nutrition* **55**, 976–980.

Kashyap, M. L., Barnhart, R. L., Srivastava, L. S., Perisutti, G., Allen, C., Hogg, E., Glueck, C. J. & Jackson, R. L. (1983). Alimentary lipemia: plasma high-density lipoproteins and apolipoproteins CII and CIII in healthy subjects. *American Journal of Clinical Nutrition* **37**, 233–243.

Kirk, R. S. & Sawyer, R. (1991). *Pearson's Composition and Analysis of Foods*, 9th ed. Harlow: Longman Scientific & Technical.

Landin, K., Holm, G., Tengborn, L. & Smith, U. (1992). Guar gum improves insulin sensitivity, blood lipids, blood pressure, and fibrinolysis in healthy men. *American Journal of Clinical Nutrition* **56**, 1061–1065.

Leadbetter, J., Ball, M. J. & Mann, J. I. (1991). Effects of increasing quantities of oat bran in hypercholesterolemic people. *American Journal of Clinical Nutrition* **54**, 841–845.

Lopes-Virella, M. F., Stone, P., Ellis, S. & Colwell, J. A. (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. *Clinical Chemistry* **23**, 882–884.

Lovegrove, J. A., Morgan, L. M., Isherwood, G. & Williams, C. M. (1993). Effect of the addition of guar gum to an acute test meal on post-prandial lipaemia in healthy young adults. *Proceedings of the Nutrition Society* **52**, 288A.

McCall, M. R., Mehta, T., Leathers, C. W. & Foster, D. M. (1992*a*). Psyllium husk I: effect on plasma lipoproteins, cholesterol metabolism and atherosclerosis in African green monkeys. *American Journal of Clinical Nutrition* **56**, 376–384.

McCall, M. R., Mehta, T., Leathers, C. W. & Foster, D. M. (1992*b*). Psyllium husk II: effect on the metabolism of apolipoprotein B in African green monkeys. *American Journal of Clinical Nutrition* **56**, 385–393.

Macdonald, C., Keyser, A. & Pacy, D. (1978). Some effects, in man, of varying the load of glucose, sucrose, fructose or sorbitol on various metabolites in blood. *American Journal of Clinical Nutrition* **31**, 1305–1311.

Mazur, A., Remesy, C., Gueux, E., Levrat, M. A. & Demigne, C. (1990). Effects of diets rich in fermentable carbohydrates on plasma lipoprotein levels and on lipoprotein catabolism in rats. *Journal of Nutrition* **120**, 1037–1045.

Meyer, H., Gomda, Y., Perez Noriega, H., Heilemann, M. & Hipp-Quarton, A. (1990). Untersuchungen uber den postprandialen Wasserstoffwechsel bei ruhenden und arbeitenden Pferden (Investigations on the postprandial water metabolism in resting and exercised ponies). In *Advances in Animal Physiology and Animal Nutrition*, no. 21, pp. 35–51 [H. Meyer, editor]. Berlin: Verlag Paul Parey.

Mirani-Oostdijk, C. P., Havekes, L., Terpstra, J., Frolich, M., Van Gent, C. M. & Jansen, H. (1983). Diurnal changes in serum triglycerides as related to changes in lipolytic enzymes, (apo)lipoproteins and hormones in normal subjects on a carbohydrate-rich diet. *European Journal of Clinical Investigation* **13**, 301–309.

Morgan, L. M. (1992). Insulin secretion and the entero-insular axis. In *Nutrient Regulation of Insulin Secretion*, pp. 1–22 [P. R. Flatt, editor]. London and Chapel Hill: Portland Press.

Nordt, T. K., Besenthal, I., Eggstein, M. & Jakober, B. (1991). Influence of breakfasts with different nutrient contents on glucose, C peptide, insulin, glucagon, triglycerides, and GIP in non-insulin-dependent diabetics. *American Journal of Clinical Nutrition* **53**, 155–160.

Ohlson, L. O., Bjuro, T., Larsson, B., Eriksson, H., Svardsudd, D., Welin, L. & Wilhelmsen, L. (1989). A cross-sectional analysis of glucose tolerance and cardiovascular disease in 67-year-old men. *Diabetic Medicine* **6**, 112–120.

Patsch, J. R., Karlin, J. B., Scott, L. W., Smith, L. C. & Gotto, A. M. (1983). Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. *Proceedings of National Academy of Sciences USA* **80**, 1449–1453.

Paul, A. A. & Southgate, D. A. T. (1978). *McCance and Widdowson's The Composition of Foods*, 4th ed. London: H.M. Stationery Office.

Pekkanen, J., Linn, S., Heiss, G., Schindran, C. M., Leon, A., Rifkind, B. M. & Tyroler, H. A. (1990). Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *New England Journal of Medicine* **322**, 1700–1707.

Pocock, S. J., Shaper, A. G. & Phillips, A. N. (1989). Concentrations of high density lipoprotein cholesterol, triglycerides, and total cholesterol in ischaemic heart disease. *British Medical Journal* **298**, 998–1002.

Rasmussen, O., Gregersen, S. & Hermansen, K. (1992). Influence of the amount of starch on the glycaemic index to rice in non-insulin-dependent diabetic subjects. *British Journal of Nutrition* **67**, 371–377.

Rillaerts, E. G., Engelmann, G. J., Von Camp, K. M. & De Leeuw, I. (1989). Effect of omega-3 fatty acids in diet of type I diabetic subjects on lipid values and hemorheological parameters. *Diabetes* **38**, 1412–1416.

Roberts, F. G., Low, A. G., Young, S., Smith, H. A. & Ellis, P. R. (1990). The effect of high viscosity guar gum flour on the rate of glucose absorption and net insulin production in the portal blood of the pig. *Proceedings of the Nutrition Society* **49**, 53A.

Schmitz, G. & Williamson, E. (1991). High-density lipoprotein metabolism, reverse cholesterol transport and membrane protection. *Current Opinion in Lipidology* **2**, 177–189.

Shutler, S. M., Bircher, G. M., Tredger, J. A., Morgan, L. M., Walker, A. F. & Low, A. G. (1989). The effect of daily baked bean (*Phaseolus vulgaris*) consumption on plasma lipid levels of young, normo-cholesterolaemic men. *British Journal of Nutrition* **61**, 257–265.

Sidery, M. B., Gallen, I. W. & MacDonald, I. A. (1990). The initial physiological responses to glucose ingestion in normal subjects are modified by a 3 d high-fat diet. *British Journal of Nutrition* **64**, 705–713.

Siedel, J., Schlumberger, S., Klose, S., Ziegenhorn, J. & Wahlefeld, A. W. (1981). Improved reagent for the enzymatic determination of serum cholesterol. *Journal of Clinical Chemistry and Clinical Biochemistry* **19**, 838–839.

Simonson, D. C. (1990). Hyperinsulinemia and its sequelae. *Hormone and Metabolic Research Supplement* **22**, 17–25.

Stinson, J. C., Owens, D., Collins, P., Johnson, A. & Tomkin, G. H. (1991). Elevation of serum insulin levels by the artificial pancreas and by oral carbohydrate loading stimulates cholesterol synthesis and storage in humans. *Proceedings of the Nutrition Society* **50**, 198A.

Tall, A. R. (1986*a*). Plasma lipid transfer proteins. *Journal of Lipid Research* **27**, 361–367.

Tall, A. R. (1986*b*). Metabolism of postprandial lipoproteins. *Methods in Enzymology* **129**, 469–482.

Taskinen, M. R. & Nikkilä, E. A. (1981). High density lipoprotein subfractions in relation to lipoprotein lipase activity of tissues in man – evidence for reciprocal regulation of HDL_2 and HDL_3 levels by lipoprotein lipase. *Clinica Chimica Acta* **112**, 325–332.

Tomkin, G. H. & Owens, D. (1991). Abnormalities of cholesterol metabolism in diabetes. *Proceedings of the Nutrition Society* **50**, 583–589.

Tredger, J., Morgan, L., Anderson, A. & Wright, J. (1991). The effect of baked bean consumption on nutrient intake and serum cholesterol levels in human subjects. *Proceedings of the Nutrition Society* **50**, 207A.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry* **6**, 24–27.

Uusitupa, M., Siitonen, O., Savolainen, K., Silvasti, M., Penttilä, I. & Parviainen, M. (1989). Metabolic and nutritional effects of long-term use of guar gum in the treatment of non-insulin-dependent diabetes of poor metabolic control. *American Journal of Clinical Nutrition* **49**, 345–351.

Yudkin, J., Alberti, K. G. M. M., McLarty, D. G. & Swai, A. B. M. (1990). Impaired glucose tolerance: is it a risk factor for diabetes or a diagnostic ragbag. *British Medical Journal* **301**, 397–402.

Zhang, J.-X., Hallmans, G., Andersson, H., Bosaeus, I., Åman, P., Tidehag, P., Stenling, R., Lundin, E. & Dahlgren, S. (1992). Effect of oat bran on plasma cholesterol and bile acid excretion in nine subjects with ileostomies. *American Journal of Clinical Nutrition* **56**, 99–105.

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