Lipid accumulation in obese Zucker rats is reduced by inclusion of raw kidney bean (*Phaseolus vulgaris*) in the diet

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The effects of inclusion of different levels of raw kidney bean (*Phaseolus vulgaris*) of high lectin content (27 g/kg meal) in a high-quality (lactalbumin) control diet were tested in nutritional trials on the growth and metabolism of obese Zucker (fafa) rats and their lean littermates in comparison with pair-fed controls. All diets contained 100 g total protein/kg and either 50 g lipids/kg (low fat) or 150 g lipids/kg (moderate fat). The growth of both obese and lean rats on bean diets was retarded by the daily bean intake in a dose-dependent manner. However, most of this was because bean-fed rats contained less body fat than the controls after 10 d. Thus, after feeding low-fat diets containing up to 130 g kidney bean/kg (lectin intake $\leq 0{\cdot}2$ g/kg body weight (BW) per d) in both 10 d and 70 d trials, the bodies of obese rats contained less fat but not protein than their pair-fed controls. Moreover, by increasing the lipid content of the diet to 150 g/kg, the level of bean inclusion could be increased to 280 g/kg (lectin intake $\geq 0{\cdot}4$ g/kg BW per d) without loss of body protein and skeletal muscle. Although these rats contained more body fat than those which were fed on low-fat diets, their weight reduction could be accounted for exclusively by reduced lipid content. In contrast, significant body protein loss occurred when the same diet of high lectin content was fed to lean littermates. Plasma insulin levels were significantly depressed in the obese Zucker rats on bean diets but the pancreas was not significantly enlarged nor its insulin content changed in 10 d trials. However, significant pancreatic growth occurred on long-term (70 d) bean feeding compared with pair-fed controls. The results suggest that, in addition to animal nutrition, it may also be possible to use the bean lectin as a dietary adjunct or therapeutic agent to stimulate gut function and ameliorate obesity if a safe and effective dose-range can be established for human subjects.

Kidney bean: Obesity: Muscle: Body composition

Obesity and associated health disorders have become an increasing worldwide problem. In addition to rising genetic predisposition to obesity in some parts of the population, many other factors, including changes in lifestyle and the diet, appear to be responsible for this development (James, 1992). It is generally believed that therapies based on animal studies aimed at counteracting hyperinsulinaemia and the consequent high rates of lipid deposition that are characteristics of most types of obesity may also benefit the long-term health of obese individuals (Proietto & Thorburn, 1994).

Oral administration of low or moderate doses of kidney bean (*Phaseolus vulgaris*) lectin, phytohaemagglutinin (PHA) to rats (up to a daily intake of 0·2 g/kg body weight (BW)) has been shown to significantly reduce both the synthesis of insulin and its secretion by the pancreas in conventional rats (Bardocz *et al.* 1996). As a consequence, moderate lipid losses occur even in young, actively growing rats but without significant increases in blood glucose levels or systemic toxic effects (Pusztai, 1991; Carvalho, 1993; Bardocz *et al.* 1996). At dietary intakes of PHA higher than 0·2 g/kg BW per d the reduction in plasma insulin levels and the consequent loss of body lipid are even more substantial. However, due to the damaging consequences of the extensive *Escherichia coli* overgrowth in the small intestine induced by the high luminal

Abbreviations: BW, body weight; CCK, cholecystokinin; NPU, net protein utilization; PAP, peroxidase–antiperoxidase; PHA, phytohaemagglutinin.

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concentration of PHA, these rats also suffer major losses of skeletal muscle and body protein (Bardocz *et al.* 1996). Thus, although it may be possible to use kidney beans, or preferably lectins, in the diet to reduce and/or prevent obesity in normal human subjects, lectin intake must be limited to relatively low doses with a consequent extension of the period of administration if potential health damage is to be avoided.

It is of considerable importance to establish whether lectins such as PHA can have similar effects in genetically obese animals. Therefore, both 10 and 70 d nutritional trials were carried out to evaluate the effects of inclusion in the diet of different levels of raw kidney beans of high lectin content on body metabolism in Zucker (fafa) rats for which hyperinsulinaemia and excessive lipid deposition are characteristic traits (Bray, 1977; Kreif & Bazin, 1991; Proietto & Thorburn, 1994). As it was thought that restricting fat intake should lead to less accretion of body fat, most of the work was initially carried out with low-fat (50 g/kg) diets. However, to compare the results with our previous work in which the diets were normalized at 150 g fat/kg diet (Bardocz *et al.* 1996), one of the trials was carried out at this moderate fat intake level.

Materials and methods

Raw kidney beans, variety 'Processor', of high lectin content, were purchased from Bucomat NV (Avelgem, Belgium). The seeds were ground in a 200 mm Glen Creston Hammermill (Glen Creston, Stanmore, Herts., UK) fitted with a 1 mm mesh. This variety has previously been chemically and nutritionally characterized (Pusztai *et al.* 1979). In the present work the PHA concentration was measured by competitive indirect ELISA (Martin-Cabrejas *et al.* 1995), and estimated to be 27 g/kg meal. Rat insulin was bought from Incstar Corp. (Stillwater, MN, USA), [^{125}I]-insulin from Amersham plc (Amersham, Bucks., UK), porcine insulin antiserum from Miles Scientific (Stoke Poges, Slough, Berks., UK) and guinea-pig serum and anti-guinea pig serum from the Scottish Antibody Production Unit (Carluke, Lanarkshire, UK). All other dietary materials and reagents were purchased from Sigma Chemical Co. (Poole, Dorset, UK).

The following isoenergetic semi-purified diets were formulated with a lipid content of 50 g/kg (low fat, LF): control diet (LFC; diet 1); 90 g bean/kg diet (LFB90; diet 2); 130 g bean/kg diet (LFB130; diet 3); 180 g bean/kg diet (LFB180; diet 4); 260 g bean/kg diet (LFB260; diet 5) and protein-free diet (LFPF; diet 6 in Table 1). In addition, two more diets were formulated which corresponded to diets normally used in previous work (Bardocz *et al.* 1996). These were of higher energy content containing 150 g fat/kg diet (moderate fat, MF) and were only used in trial 2: control (MFC; diet 7) and 260 g bean/kg diet (MFB260; diet 8). With the exception of the protein-free diet, all diets contained a total of 100 g protein/kg diet derived either from lactalbumin alone or a combination of kidney-bean meal and lactalbumin. Mineral and vitamin mixtures used have been described previously (Grant *et al.* 1993) and the diets were supplemented with individual amino acids to bring their overall profile up to the amino acid requirements for rats (Coates *et al.* 1969).

Animal Management

All management and experimental procedures were carried out in strict accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 by staff licensed to carry out such procedures.

Animals

Male obese (fafa) or lean (FaFa or faFa) Zucker rats, reared and housed in the small-animal unit of the Rowett Research Institute, were weaned at 21 d and given free access to a non-purified stock diet (Labsure, Manea, Cambs., UK) for

Table 1. Components of the diets used in Zucker experiments (g/kg diet)

Diet no. ...	1	2	3	4	5	6	7	8
Diet ...	Control; LFC	Bean; 90 g/kg; LFB90	Bean; 130 g/kg; LFB130	Bean; 180 g/kg; LFB180	Bean; 260 g/kg; LFB260	Protein-free; LFPF	Control; MFC	Bean; 260 g/kg; MFB260
Lactalbumin	120	100	90	78	60	0	120	60
Kidney bean	0	90	130	180	260	0	0	260
Maize starch	443	372	341	303	240	563	380	177
Potato starch	100	100	100	100	100	100	100	100
Glucose	112	112	112	112	112	112	150	150
Maize oil	50	50	50	50	50	50	150	150
Vitamins	50	50	50	50	50	50	50	50
Minerals	50	50	50	50	50	50	50	50
Glycerol	75	75	75	75	75	75	0	0
L-Tryptophan	0	0·12	0·14	0·20	0·28	0	0	0·28
L-Methionine	0	0·73	1·08	1·52	2·15	0	0	2·15
Silicic acid	0·40	0·40	0·40	0·40	0·40	0·40	0·40	0·40
Lectin*	0	2·4	3·5	4·8	7·0	0	0	7·0
Enzyme inhibitor†	0	0·3	0·4	0·6	0·9	0	0	0·9

* Grant *et al.* (1993).
† Gram equivalents of Bowman–Birk inhibitor (Grant *et al.* 1995).

14–18 d. They were then individually housed and fed on a powdered lactalbumin-based control diet (diet 1 in Table 1) for 9 d. Initially, the control diet was freely available but from day 4 onwards the amount of food offered was restricted to 10 g/rat per d. This was offered as three meals: 2·5 g at 09.00 hours, 2·0 g at 13.00 hours and 5·5 g at 17.00 hours. Water was freely available at all times. On day 9 the rats were given 2·5 g control diet at 09.00 hours and a blood sample from the tail vein was collected in a heparinized tube exactly 2 h later, centrifuged after the addition of plastic granules and the supernatant fraction used to measure insulin and glucose levels of the rats before commencing the experiments. This procedure (Bardocz *et al.* 1996) was used throughout the present study. Rats were then given a further 2·0 g control diet at 13.00 hours and 5·5 g at 17.00 hours before the start of the experiments.

Trial 1

Groups of Zucker (fafa) rats (44–48 d old, five rats randomly selected for each diet) were fed for 10 d on one of the following low-fat diets: control diet (diet 1), a protein-free diet (diet 6) or a kidney-bean diet which contained either 90, 130, 180 or 260 g kidney-bean meal/kg (diets 2–5 in Table 1). Each rat was given 10 g diet offered as three feeds as before. This amount of food closely approximated the average daily intake of these rats when given free access to the LFB260 diet ensuring that all the diet was consumed by the rats. This intake was over 50 % of that consumed by rats given free access to a control diet but was well above that necessary to meet their minimum requirements for energy, vitamins and minerals. Net protein utilization (NPU) was determined as described before (Pusztai *et al.* 1979).

On day 9, the rats were given 2·5 g of the appropriate diets at 09.00 hours and a blood sample was collected from the tail vein 2 h later. The rats were then fed as normal for the rest of the day. On day 10, the rats were given 2·5 g of the appropriate diet and killed 2 h later by anaesthetic (halothane) overdose followed by exsanguination.

The stomach, small intestine, caecum and colon were removed and the contents flushed out with ice-cold distilled water. The pancreas, spleen, liver, kidneys, thymus, lungs, heart and gastrocnemius hind-limb muscles were also excised. The tissues and remaining carcasses were frozen on dry ice, weighed, freeze-dried and reweighed.

Trial 2

Two groups of randomly selected obese Zucker rats (fafa) pre-treated as in trial 1 (44–46 d old; five rats in each group) were fed 9·5 g of a low-fat or a moderate-fat bean diet containing 260 g kidney-bean meal/kg diet (LFB260 or MFB260) for 10 d. Two control groups were also fed for 10 d on 9·5 g low-fat or moderate-fat control diets (LFC or MFC). In addition, two groups of lean Zucker littermates (FaFa or Fafa) were also fed for 10 d on 9·5 g either the moderate-fat control (MFC) or bean diets containing 260 g kidney bean/kg (MFB260). Killing and subsequent procedures were the same as in trial 1.

Trial 3

Obese Zucker rats (fafa) were weaned at 21 d and fed *ad libitum* on stock diet for 10–14 d after which they were pre-fed 10 g lactalbumin diet for 9 d. The rats were then randomly selected into two groups of ten rats each and were fed on either low-fat control diet (LFC) or a diet containing 130 g kidney-bean meal/kg diet (LFB130) for 70 d. Initially, each rat was offered 10 g diet/d given as three feeds as before. This was increased to 13 g after 7 d, 15 g after 14 d and to 17 g after 22 d. From day 30 onwards the amount of food offered was 18 g/rat per d (4·5 g at 09.00 hours, 3·0 g at 13.00 hours and 10·5 g at 17.00 hours). All other procedures were as for trial 1.

Analysis

Appropriate dried tissues and carcass samples were combined and ground in a mincer. Lipid was extracted from the ground material with solvent (1 : 100, w/v), the solvent being chloroform–methanol (2 : 1, v/v) as before (Grant *et al.* 1989). N estimations were done on the defatted carcass material using a Foss Heraeus Macro N automated system (Foss (Electric) UK, Bishopthorpe, Yorks., UK). Protein content was calculated as N × 6·25. Measurement of immunoreactive serum insulin by radioimmunoassay using rat insulin as a standard, and interassay CV were as described before (Bardocz *et al.* 1996). Glucose was determined by the glucose oxidase (*EC* 1.1.3.4) method (Trinder, 1967) using an autoanalyser. Interassay CV for ten assays was 6·5 % and remained unchanged over a 2 year period. Pancreatic insulin was extracted according to the procedure of Melmed *et al.* (1973).

Histology

Pieces of jejunum (20 mm; 50 mm from pylorus) were fixed in 40 g/l buffered (pH 7) paraformaldehyde, embedded in paraffin wax and sections (3 μm) were stained with haematoxylin and eosin for morphological measurements. Ten properly oriented jejunal crypts were selected at random from each animal and their length measured. Results were calculated as means and standard deviations for five rats per treatment group.

Antibody-peroxidase–antiperoxidase (PAP) staining of the sections was carried out as before (Pusztai *et al.* 1990). Briefly, after inhibition of the endogenous peroxidase in fixed tissue sections, antigenic sites were unmasked by trypsinization and the sections were reacted with appropriate anti-PHA antibodies in the presence of fetuin, followed by the link antiserum and then PAP serum. The bound PHA lectins were then visualized with 3,3′-diaminobenzidine and the sections were counter-stained with haematoxylin.

Statistics

One-way ANOVA was performed on the data using the Minitab statistical software package (Minitab, New York, NY, USA) and multiple comparisons were done by the

Tukey test using the Instat statistical package (Graphpad Software Inc., San Diego, CA, USA). Results were expressed as arithmetic means with their pooled standard deviation.

Results

Trial 1

Inclusion of kidney bean in low-fat diets of obese Zucker rats caused significant changes in their growth, body composition, tissue weights and hormone balance (Tables 2 and 3). Thus, obese rats given a low-fat diet containing 260 g kidney bean/kg (LFB260, diet 5; Table 1) for 10 d (10 g diet/rat per d; lectin intake: 0·4 g/kg BW per d) lost weight and their dry body weight became significantly less by the end of the experiment. Similarly, the dry body weight of rats fed on the 180 g kidney bean/kg diet (0·28 g lectin/kg BW per d; LFB180, diet 4 in Table 1) was also less after 10 d than that of pair-fed controls. However, this reduction was slight and in fact the rats maintained their initial fresh body weight. In comparison, pair-fed control (LFC, diet 1 in Table 1) rats gained on average about 0·7 g fresh BW/d and almost 0·4 g dry BW/d (Table 2). Obese rats fed on bean diets always ended up with less body fat than the controls and this accounted for most of their weight loss. However, with the LFB260 diet, but not the LFB180 diet, there was also a highly significant loss of muscle and body protein (Table 2).

The fresh-weight gain of obese Zucker rats given the low-fat diet containing 130 g kidney bean/kg (LFB130, diet 3 in Table 1; lectin intake: 0·2 g/kg BW per d) was not significantly different from that of the pair-fed controls over the 10 d (Table 2). Moreover, their skeletal (gastrocnemius) muscle weights were not altered and their carcass protein content was also similar to that of controls. However, their dry weight was significantly lower because they deposited essentially no body fat in 10 d (Table 2).

Consumption of a low-fat diet containing 90 g kidney bean/kg (LFB90) for 10 d did not significantly affect the growth or body composition of the obese Zucker rats although their body lipid content was reduced slightly compared with controls (Table 2).

NPU by obese Zucker rats was poor even in animals given the control lactalbumin-containing diet (Table 2). It was not significantly affected by inclusion of up to 180 g kidney bean/kg diet but was reduced at higher levels of inclusion.

Small-intestinal weights were elevated in a dose-dependent manner in all obese Zucker rats given bean diets (Table 2). Moreover, the LFB260 diet potently stimulated crypt enlargement, regardless of whether it was incorporated in low-fat or moderate-fat diets. Thus, the mean crypt sizes in kidney-bean-fed rats given low-fat and moderate-fat diets were 195 (SD 3) and 211 (SD 4) μm, while the appropriate control values were 87 (SD 2) and 92 (SD 2) μm respectively (results from Plate 1). Moreover, PHA was shown by the PAP technique to bind avidly to the surface of small-intestinal villi (Plate 1). Colon weights were elevated only in obese rats with the LFB260 diet.

Neither the weight nor the insulin content of the pancreas of obese Zucker rats was affected by inclusion of kidney bean in their diets for 10 d (Tables 2 and 3). However, circulating insulin levels were reduced in rats given bean diets (results given for LFB260 and LFB130 diets) but without significant changes in glucose levels (Table 3).

Trial 2

The nutritional effects of feeding obese rats on kidney-bean diets of low or moderate fat content were significantly different (Table 4). Although lipid deposition was higher on moderate than on low-fat diets, the obese rats fed on the moderate fat MFB260 diet went on depositing body protein at a rate that was at least as good or better than that of the control rats given the MFC diet, in contrast to the zero

Table 2. Initial and final fresh body weights (FBW), final dry body weights (DBW), body lipid and protein contents, net protein utilization (NPU) values and tissue weights (mg/100 g lipid-free BW) of obese Zucker rats fed on low-fat (50 g/kg) diets containing various levels of kidney bean, a control diet or a protein-free diet for 10 d*†

Diet no. ... Diet...	1 LFC	2 LFB90	3 LFB130	4 LFB180	5 LFB260	6 LFPF	Pooled SD
Lectin intake (g/kg BW per d)	0	0·14	0·20	0·28	0·40	0	
Initial FBW (g)	175·2[a]	175·4[a]	174·4[a]	178·7[a]	177·6[a]	173·9[a]	5·0
Final FBW (g)	182·2[a]	181·2[a]	180·0[a]	178·2[a]	163·2[b]	160·9[b]	5·5
Final DBW (g)	88·22[a]	86·70[a]	84·41[b]	82·17[c]	76·03[d]	81·20[c]	1·51
Lipid (g)	50·23[a]	49·24[a]	46·95[b]	44·82[c]	41·00[d]	49·95[a]	1·52
Protein (g)	27·02[a]	26·93[a]	27·00[a]	26·06[a]	25·43[b]	21·72[b]	0·84
NPU	50·5[a]	54·8[a]	55·8[a]	44·2[a]	37·9[b]	–	9·3
Small bowel (mg/100 g lipid-free BW)	3282[a]	3915[b]	4334[bc]	4733[c]	5050[c]	2980[a]	300
Colon (mg/100 g lipid-free BW)	400[a]	460[a]	477[a]	500[a]	533[b]	440[a]	60
Pancreas (mg/100 g lipid-free BW)	699[a]	718[a]	711[a]	728[a]	711[a]	600[b]	43
Gastrocnemius (mg/100 g lipid-free BW)	739[a]	692[a]	696[a]	656[b]	565[c]	546[c]	35

[a,b,c] Values within a row not sharing a common superscript letter were significantly different, $P \leq 0.01$.
* All groups were given 10 g diet/rat per d. Initial values for Zucker rats before feeding were: DBW 84·3 (SD 0·9) g, lipid 46·9 (SD 0·8) g, protein 26·7 (SD 0·3) g.
† For details of diets, see Table 1.

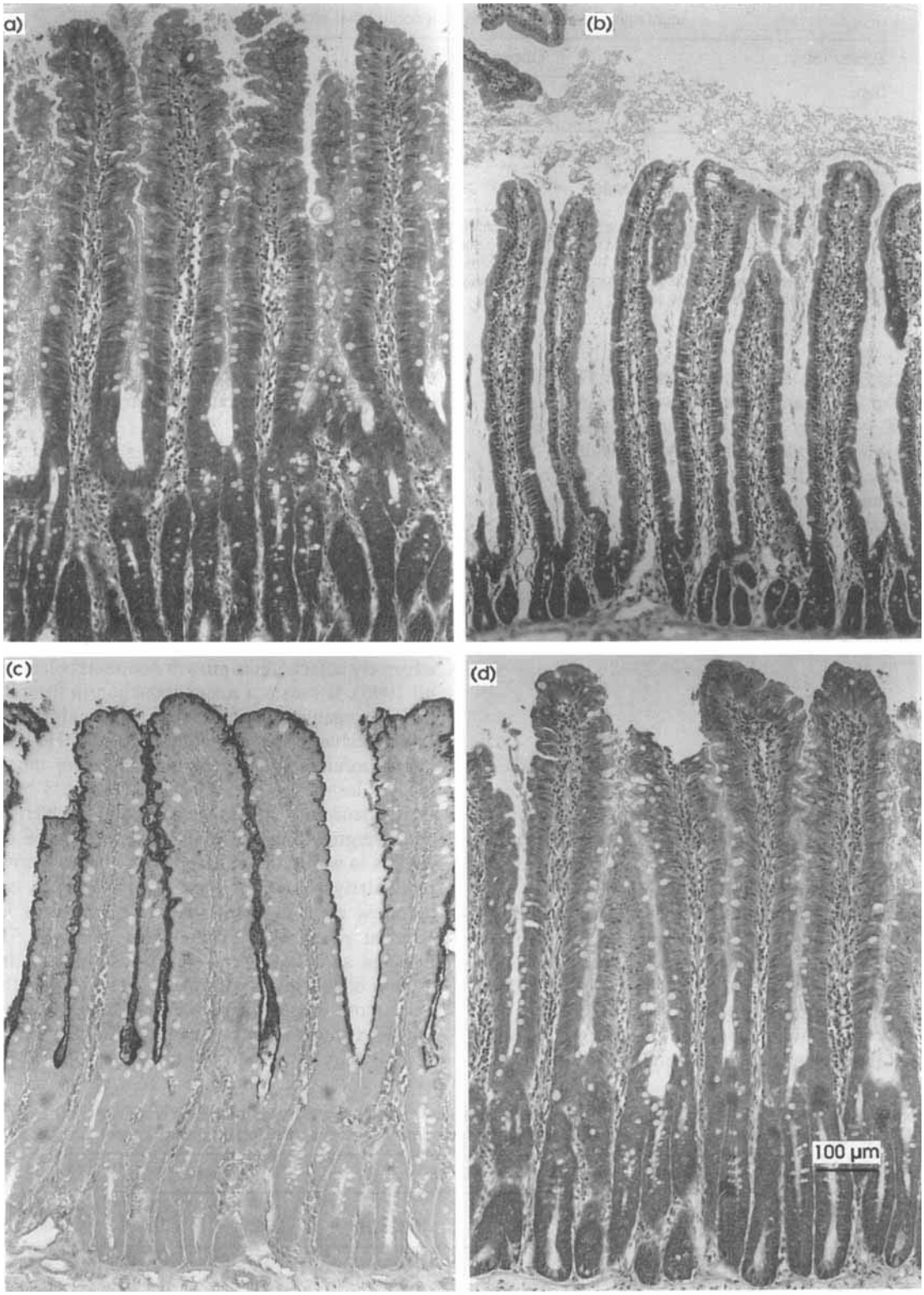

Plate 1. Light microscope sections of paraformaldehyde-fixed small-intestinal tissue (after staining with haematoxylin and eosin) of obese Zucker rats fed for 10 d on (a) kidney bean diet (LFB260), and (b) control (LFC) diet respectively. Sections (c) and (d) were also from the small intestine of rats fed on LFC260 diet for 10 d. Section (c) was first reacted with monospecific anti-phytohaemagglutinin rabbit antibody and this was followed by second antibody-peroxidase–antiperoxidase (PAP) staining; section (d) is the appropriate control which was reacted with rabbit pre-immune serum instead of the anti-phytohaemagglutinin antibody but followed by the same PAP methodology.

Table 3. Comparison of serum insulin and glucose levels in obese and lean Zucker rats fed on diets containing raw kidney bean or a control diet after 9 d*†

Zucker rat...	Obese			Lean		
Diet...	1 LFC	3 LFB130	5 LFB260	7 MFC	8 MFB260	Pooled SD
Pancreas insulin (μg)	80·9^{c}	60·5^{c}	57·9^{c}	40·6^{b}	22·4^{a}	18
Serum insulin (ng/ml)	20·0^{d}	11·0^{c}	7·0^{b}	7·0^{b}	2·0^{a}	2·1
Serum glucose (mg/ml)	1·98^{a}	1·77^{a}	1·44^{a}	1·75^{a}	1·70^{a}	0·30

^{a,b,c,d} Values within a row not sharing a common superscript letter were significantly different, $P < 0·01$.
* Initial serum levels for obese rats were: 21·2 (SD 2·5) ng insulin/ml and 1·78 (SD 0·19) mg glucose/ml; for lean (Zucker) rats: 6·5 (SD 0·6) ng insulin/ml and 1·80 (SD 0·15) mg glucose/ml. To avoid potential interference by halothane overdose, serum analyses were carried out on samples taken on the 9th day.
† For details of diets, see Table 1.

protein accretion in rats fed on the low-fat LFB260 diet. However, when the lean Zucker rats were given diets containing 260 g kidney bean/kg, regardless of whether these were of low or moderate fat content (results in Table 4 are only given for MFB260), considerable weight loss occurred over the 10 d experimental period, including both body lipid and protein losses. These lean Zucker rats fed on the MFB260 diet for 10 d had significantly higher pancreas weight (597 mg/100 g lipid-free BW) than the control rats (472 mg/100 g lipid-free BW; $P < 0·01$). However, as with the obese rats, their serum insulin, but not glucose, levels were reduced (Table 3).

Trial 3

In longer-term feeding experiments obese Zucker rats fed on low-fat diets containing 130 g kidney bean/kg diet (LFB130) gained weight (approximately 1·5 g fresh weight and 1·3 g dry weight/d) throughout the 70 d experimental period (Table 5) but at a lower rate than the controls (approximately 2·4 g fresh weight and 1·8 g dry weight/d). The bean-fed rats deposited just under 1·0 g lipid and 0·2 g protein/d whereas control animals accumulated close to 1·5 g lipid and slightly more than 0·2 g protein/d. As a result, the carcass lipid content of rats fed 130 g kidney bean/kg diet was significantly lower after 70 d (Table 5). Although body protein content and the weight of the gastrocnemius muscle in these rats were slightly reduced, the changes were not significant. The weights of the small intestine, colon and pancreas were, however, significantly increased (Table 5).

Discussion

As shown previously, the inclusion of high amounts of kidney bean PHA, its main physiologically active lectin component, in the diet of young actively growing conventional rats at levels of 0·4 g/kg BW per d or more, adversely affects their growth and metabolism (Bardocz *et al.* 1996). It causes a major reduction in their body fat but the accompanying high catabolic protein loss makes this an unacceptable means of weight reduction. However, PHA is not harmful for germ-free rats, therefore the weight and muscle losses are not direct lectin effects but rather the consequence of the damage caused by the PHA-induced *E. coli* overgrowth in the small intestine (Pusztai *et al.* 1993). As this is negligible at daily PHA intakes below 0·2 g and particularly below 0·1 g lectin/kg BW, PHA is essentially harmless even in conventional rats at such low intakes (Pusztai *et al.* 1993; Bardocz *et al.* 1996). Thus, PHA behaves as a biological signal that induces hyperplastic growth of the small intestine, hypertrophy of the pancreas and, most importantly, reduces body lipid deposition. Indeed, conventional rats continue to gain weight on diets of low PHA content for up to 2 years (Grant *et al.* 1995).

Table 4. Body weights and composition of Zucker rats fed on diets containing raw kidney bean or a control diet of moderate fat (150 g/kg; MF) content for 10 d in comparison with rats fed on similar diets containing 50 g lipid/kg diet (low fat; LF)*†

Zucker rat....	Lean		Obese		Obese		
Diet no. ...	7 MFC	8 MFB260	7 MFC	8 MFB260	1 LFC	5 LFB260	Pooled SD
Initial FBW (g)	172·1^{a}	170·5^{a}	170·0^{a}	169·6^{a}	170·6^{a}	168·5^{a}	5·8
Final FBW (g)	184·5^{a}	165·1^{b}	185·8^{a}	164·9^{b}	176·8^{d}	152·4^{e}	4·2
Final DBW (g)	63·23^{a}	46·37^{b}	95·00^{c}	80·50^{d}	92·09^{c}	73·62^{e}	4·11
Lipid (g)	11·99^{a}	3·96^{b}	57·78^{c}	44·27^{d}	54·68^{c}	39·92^{e}	2·30
Protein (g)	44·91^{a}	37·14^{b}	27·44^{c}	28·15^{c}	27·17^{c}	25·99^{c}	1·43

FBW, fresh body weight; DBW, dry body weight.
^{a,b,c,d,e} Values within a row not sharing a common superscript letter were significantly different, $P < 0·01$.
* The daily intake of rats was 9·5 g diet. For details of diets and procedures, see Table 1 and p. 215. The lectin intake of rats given kidney bean was 0·4 g/kg body weight per d.
† Initial values for the Zucker rats before feeding were: obese, DBW 81·9 (SD 0·9) g; lipid 45·5 (SD 0·6) g; protein 26·0 (SD 0·5) g; lean, DBW 58·9 (SD 1·1) g; lipid 11·2 (SD 1·1) g; protein 41·7 (SD 0·7) g.

Table 5. Body weights (BW) and composition, changes in body composition and tissue weights (mg/100 g lipid-free BW) of obese Zucker rats fed on a low-fat (50 g/kg) kidney-bean-based diet or a control diet (pair-fed) for 70 d*†

Diet no. ...	1	3	
Diet...	LFC	LFB130	Pooled SD
Initial BW (g)	145·7[a]	146·6[a]	8·0
Final BW (g)	315·0[a]	251·6[b]	14·0
Dry BW (g)	198·4[a]	160·3[b]	6·8
Lipid (g)	138·5[a]	105·2[b]	5·2
Protein (g)	39·1[a]	35·2[a]	4·0
Small intestine (mg/100 g lipid-free DBW)	2817[a]	4869[b]	350
Colon (mg/100 g lipid-free DBW)	400[a]	505[b]	51
Pancreas (mg/100 g lipid-free DBW)	460[a]	701[b]	84
Gastrocnemius (mg/100 g lipid-free DBW)	626[a]	600[a]	61

DBW, dry body weight.
[a,b] Values within a row not sharing a common superscript letter were significantly different, $P < 0.01$.
* For details of diets and procedures, see Table 1 and p. 215.
† Initial values for the Zucker rats before feeding were: DBW 70·8 (SD 0·9) g, lipid 38·2 (SD 0·9) g, protein 22·6 (SD 0·5) g. Initially each rat was offered 10 g diet/d given as three feeds. This was increased to 13 g after 7 d, 15 g after 14 d and 17g after 22 d. From day 30 onwards the amount of food offered was 18 g/rat per d (4·5 g at 09.00 hours, 3·0 g at 13.00 hours and 10·5 g at 17.00 hours).

Obese Zucker rats have a much higher body lipid content and plasma insulin level than their lean littermates or normal rats of the same age and weight. Despite this genetic predisposition to obesity, their fat deposition was significantly reduced by feeding them on diets containing moderate levels of kidney bean, without unacceptable muscle loss. Indeed, at comparable PHA intakes body and muscle protein catabolism was considerably less in obese rats than in their lean littermates or conventional rats. Thus, a daily intake of 0·2 g lectin/kg BW with the LFB130 diet with mild antinutritional effects in conventional rats (Bardocz *et al.* 1996), was well tolerated by the obese rats with substantial reduction in body lipid (Table 2) but no significant loss of body protein or muscle regardless of whether the diet contained low or moderate fat. Moreover, even at the highest lectin intake (≥0·4 g/kg BW per d), particularly with medium-fat diets, the weight and protein losses in obese rats were similar to pair-fed controls and significantly less than those in lean littermates or conventional rats (Table 4). This was probably because lipid deposition is predominant in the obese rat, whereas both protein and lipid deposition occur at high rates in lean or conventional animals (Proietto & Thorburn, 1994). These differences in final body composition after 10 d on different diets are highlighted in Figs. 1 and 2.

The inclusion of kidney bean in the diet also helped to reduce fat deposition in obese rats in long-term feeding. Although only one kidney-bean concentration was tested in trial 3 (*LFB130* diet), the obese rats fed on this diet for 70 d deposited one-third less fat than their pair-fed controls and this accounted for about 76% of the difference in dry BW between bean-fed and control rats. The potential usefulness of this long-term feeding strategy to reduce fat accretion was further underlined by the finding that their body protein

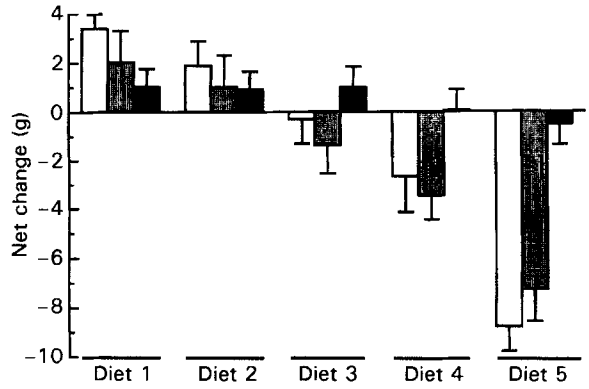

Fig. 1. Differences in final dry body weight (□) and body lipid (▨) and protein (■) contents of obese Zucker rats fed on low-fat (50 g/kg) control or kidney-bean diets for 10 d. The concentration of kidney bean varied from zero (control) to 260 g kidney bean/kg diet. For details of diets, see Table 1.

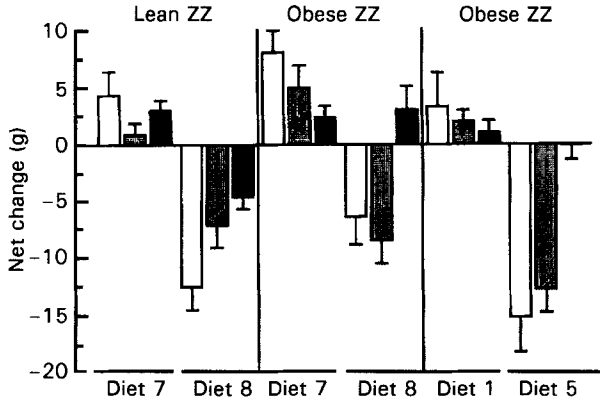

Fig. 2. Differences in final dry body weight (□) and body lipid (▨) and protein (■) contents of lean and obese Zucker rats (ZZ) fed for 10 d on diets of moderate (150 g/kg) or low (50 g/kg) fat content, and containing 0 or 260 g kidney bean/kg diet. For details of diets, see Table 1.

content did not differ significantly after 70 d of feeding (Table 5).

The results clearly indicate that kidney bean or PHA can, at least in part, override the genetic predisposition of the obese Zucker rat to deposit lipid, possibly because its presence in the diet leads to the depression of insulin levels in blood circulation (Table 3). However, as the insulin content of the pancreas of obese rats was unchanged after 10 d, its synthesis was not affected. Thus, PHA probably only interfered with the secretion of insulin by an unknown mechanism. This is unlike the situation in normal rats in which the insulin level was also reduced in the pancreas (Pusztai *et al.* 1991; Carvalho, 1993; Bardocz *et al.* 1996). However, this difference in response may also reflect differences in the expression levels of pancreatic and peripheral hormone receptors (Knott *et al.* 1992) and/or the responsiveness to regulatory hormones of obese and lean rats.

The precise mechanism of the lectin effect on insulin secretion is unknown. However, lectins survive the passage through the alimentary tract in intact and fully reactive form, bind to small-intestinal epithelial cells, including

enteroendocrine cells (King *et al.* 1980; Pusztai *et al.* 1995), and trigger the release of CCK, glucagon and possibly other gut regulatory peptides (Grant *et al.* 1987; Pusztai, 1991; Carvalho, 1993; Banwell *et al.* 1995; Herzig *et al.* 1997). It is, therefore, possible that the interference with insulin secretion is an indirect effect of PHA and that changes in the metabolic balance between lipid deposition and catabolism on one hand, and between muscle protein synthesis and degradation on the other, are due to the action of the secreted regulatory peptides in modulating the levels of hormones and their peripheral receptors.

PHA is a potent *in vitro* mimic of insulin (Pusztai & Watt, 1974). As it is endocytosed by small-bowel enterocytes *in vivo*, traverses the gut wall and is subsequently released into the systemic circulation (Pusztai *et al.* 1989), PHA may also directly interfere with the effects of insulin on body metabolism. By binding to hormone receptors on key organs and tissues, such as the insulin receptors on fat cells (Pusztai & Watt, 1974) and muscle cells (Bardocz *et al.* 1993), pancreatic CCK receptors (Grant *et al.* 1997) and others, PHA may directly trigger changes in metabolism. However, these need not necessarily be similar to those occurring *in vitro*. Thus, pancreatic CCK receptors of the obese Zucker rat appear to be of low affinity or low in number or both and therefore can be less responsive to CCK than those of the conventional rat (McLaughlin *et al.* 1982). This may, in fact, be the reason for the relatively abnormal pancreatic metabolism in obese rats as the weight of their pancreas did not increase significantly after feeding either the control or kidney-bean diet for 10 d, in contrast to the rapid pancreatic growth of young conventional rats (Pusztai *et al.* 1992; Herzig *et al.* 1997). Furthermore, although the pancreas did grow in obese rats given the kidney-bean diet for 70 d, the response differed from that in conventional rats where the trophic effects of PHA on the pancreas diminished with duration of the feeding and ageing of the rat (Grant *et al.* 1993, 1995).

One of the major findings of the present work was that kidney-bean diets had as potent a growth factor effect on the small intestine of obese rats as on conventional animals (Pusztai *et al.* 1990, 1992), probably as a result of the avid binding to and endocytosis of PHA by brush-border epithelial enterocytes (Plate 1). The growth was by hyperplasia as shown by the substantial (over 2·2-fold) enlargement of the crypts of Lieberkhün, probably with a high nutritional cost. In fact, increased sequestration of nutrients by the small bowel could be a contributory factor to reducing body fat levels in the obese rat. The higher than normal nutrient uptake of the stimulated gut is facilitated by the decrease in fat deposition due to the low insulin levels and also by the growth signal-induced redirection of the flow of dietary nutrients from deposition to the growing small bowel. However, it is unclear at present how this occurs and even less is known about possible metabolic reactions which can link the stable blood glucose, the low insulin levels and the reduced deposition and/or increased catabolism of fat to the nutrient support of gut growth, particularly as fatty acids are normally not directly involved in this growth.

In conclusion, this study demonstrated that the inclusion of kidney bean in the diet leads to a substantial reduction in fat deposition and/or mobilization of body fat in both obese and lean Zucker rats. This was achieved without body and muscle protein losses in obese rats, even at high doses of kidney bean; this was not the case for the lean littermates. These results provide a firm basis for exploring the nutritional and/or pharmacological use of kidney bean or PHA to induce small-bowel growth, reduce hyperglycaemia and obesity in animals and possibly in man, though safe and effective dose ranges will have to be established for each species.

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