Immunoreactive gastric inhibitory polypeptide and K cell hyperplasia in obese hyperglycaemic (ob/ob) mice fed high fat and high carbohydrate cafeteria diets

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Abstract. The effect of diet composition on plasma and intestinal concentrations of immunoreactive gastric inhibitory polypeptide (GIP) and intestinal K cell density was examined in obese hyperglycaemic (ob/ob) mice. The mice were reared from 3 to 11 weeks of age on either stock diet, a high fat (HF) cafeteria diet or a high carbohydrate (HC) cafeteria diet. The HF cafeteria diet increased the concentration of GIP in plasma (75%) and in the intestine (118%) and increased the density (54%) of GIP-secreting K cells in the upper jejunum compared with the stock diet. Plasma and intestinal GIP concentrations were not significantly altered by the HC cafeteria diet, although the density of K cells in the upper jejunum was increased (45%). The extent of hyperglycaemia and hyperinsulinaemia in ob/ob mice was not significantly altered by the HF and HC cafeteria diets. The results indicate that an increased amount of dietary fat chronically stimulates the production and secretion of GIP, and enhances intestinal K cell density in ob/ob mice.

Obese hyperglycaemic (ob/ob) mice exhibit a particularly severe obesity and hyperinsulinaemia, marked hyperphagia and moderate hyperglycaemia (Bray & York 1979; Bailey et al. 1982). Recent studies have identified excessive concentrations of immunoreactive gastric inhibitory polypeptide (GIP) in the small intestine and plasma of these mice (Flatt et al. 1983, 1984a) associated with hyperplasia and increased hormone content of the intestinal GIP-secreting K cells (Polak et al. 1975). Considerable evidence has implicated GIP as a physiological component of the enteroinsular axis (Brown 1982; Creutzfeldt et al. 1983), and hypersecretion of GIP has been suggested as a factor contributing to the hyperinsulinaemia of ob/ob mice (Flatt et al. 1984a,b). The raised GIP concentrations of ob/ob mice render this mutant a convenient model for studies of GIP physiology. As noted in other species (Brown 1982), orally administered fat elicits a greater acute plasma GIP response than other nutrients in ob/ob mice (Flatt et al. 1984a), but the long-term effects of different dietary components on plasma and intestinal GIP remain to be established.

The present study examines the chronic effects of excess fat and excess carbohydrate on the development of raised GIP concentrations in young ob/ob mice fed for 8 weeks on high fat and high carbohydrate cafeteria diets.

Materials and Methods

Animals

Obese hyperglycaemic (ob/ob) mice and lean (+/+ ) mice on the Aston background were housed as previously (Flatt et al. 1984a). The origin and characteristics of these mice have been described elsewhere (Flatt & Bailey 1981; Bailey et al. 1982).

Diets

Groups of mice were fed either stock diet (Mouse breeding diet, Heygate & Sons Ltd., Northampton, UK)
or approximately isoenergetic high fat (HF) and high carbohydrate (HC) cafeteria diets. The diets and tap water were available ad libitum. The metabolisable energy contents of the diets were: stock diet 12.2 MJ/kg (26% protein, 8% fat, 66% carbohydrate); HC cafeteria diet 13.2 MJ/kg (10% protein, 7% fat, 83% carbohydrate). The HF and HC cafeteria diets were supplied in the form of 2 food items daily. Five pairs of food items were used in rotation in each cafeteria diet. The pairs of HF cafeteria foods were chocolate and fish cake, fish paste and corned beef, sardines and pork pie, marzipan and roast beef, beefburger and sausage. For the HC cafeteria diet the food pairings were white bread and matzo, rice krispies breakfast cereal and malt bread, cornflakes breakfast cereal and jelly, sugar puffs and weetabix breakfast cereals, madeira cake and rye crispbread (Bailey et al. 1985). Nutrient composition and metabolisable energy values were calculated from food tables (Paul & Southgate 1978) or manufactures information.

**Experimental procedure**

Three groups of ob/ob mice were matched for sex and body weight immediately prior to weaning (3 weeks of age), and weaned onto either the stock diet, HF or HC cafeteria diet. A group of lean mice was also weaned onto the stock diet. The mice were maintained on their respective diets until 11 weeks of age, when 24 h food intake was monitored (Bailey et al. 1985) for 4 consecutive days. Body weight was recorded, and a blood sample (150 µl) was taken from the tail tip for determination of plasma glucose, insulin and GIP.

Mice were killed by cervical dislocation at 12 weeks of age. The intestine (duodenum-jejunum-ileum) was rapidly removed, cleaned by perfusion with ice cold saline, weighed and the length measured. One cm segment was taken exactly 15 cm distal to the duodenal-pyloric junction (corresponding to upper jejunum), fixed for 24 h in Bouin’s fluid, dehydrated through graded ethanols, cleared in toluene and embedded in paraffin wax. The remaining tissue was re-weighed, extracted with 5 ml/g acid ethanol (Flatt et al. 1983) and assayed for GIP.

**Assay**

Plasma glucose was measured by an automated glucose oxidase procedure (Stevens 1971), and plasma insulin was determined by double antibody radioimmunoassay using crystalline mouse insulin standard (Bailey & Ahmed-Sorour 1980). GIP was measured by double antibody radioimmunoassay (Morgan et al. 1978) using donkey anti-rabbit gamma globulin antiserum (Guildhay Antisera, University of Surrey, Guildford, UK) to separate bound and free antigen. Immuno-adsorbed hormone-free plasma was used to minimise non-specific interference, and parallelism was demonstrated between the standard curve and serially diluted ob/ob mouse plasma. Porcine GIP (J. C. Brown, University of British Columbia, Canada) was used to prepare 125I-labelled tracer and as standard. The GIP antiserum (RIC 34/III, Guildhay Antisera), raised in rabbit against a porcine GIP-glutaraldehyde-ovalbumin conjugate, recognises the 5000 and 8000 molecular forms of GIP, and exhibits negligible cross-reactivity with other enteropancreatic hormones. Details of the assay sensitivity and specificity have been described previously (Morgan et al. 1978; Flatt et al. 1984a).

**Immunocytochemistry**

Rehydrated paraffin sections (5 µm) were cut transversely from the segments of upper jejunum. The sections were immunostained by the unlabelled peroxidase-antiperoxidase (PAP) technique (Sternberger 1979) using rabbit anti-porcine GIP antiserum (RIC 34/III) described above. Sections were incubated with the antiserum (1:1500 dilution) for 16 h at 4°C, followed by incubations with donkey anti-rabbit antiserum (Guildhay Antisera) for 40 min at 4°C and with rabbit PAP complex (Dakopatts, Glostrup, Denmark) for 40 min at 4°C. Peroxidase activity was visualised using 0.05% 3,3′-diaminobenzidine (British Drug Houses, Poole, UK) in citrate-acetate buffer, pH 5, and sections were counterstained with 0.5% Harris’ haematoxylin (British Drug Houses, Poole, UK). Control sections were treated with normal rabbit serum instead of rabbit anti-porcine GIP antiserum. Sections were washed twice for 10 min with saline in 0.05 M Tris buffer, pH 7.6, between each incubation. Thirty-five representative sections were selected from throughout each tissue segment. Positively stained GIP cells (K cells) were counted per whole transverse section at × 250 magnification. Sections were focused up and down to ensure inclusion of all cells throughout the thickness of the sections. Only sections with villi at right angles to the lumen were used, and only cells with a surface area equal to or greater than a nucleus were counted. The area of each section was determined using a grid, and the number of K cells was expressed per mm², to accommodate variations in area.

**Statistical analysis**

Data were compared using Student’s unpaired t-test. Differences were considered to be significant for P < 0.05.

**Results**

At 11 weeks of age ob/ob mice fed stock diet were characteristically obese (as indicated by greater body weight), hyperphagic, hyperglycaemic, hyperinsulinaemic and exhibited raised plasma GIP concentrations compared with lean mice fed the same diet (Fig. 1). The ob/ob mice fed a HF
cafeteria diet, but not those fed a HC cafeteria diet, showed a greater body weight than ob/ob mice fed a stock diet. However, energy intake during the last 4 days of the study was similar in ob/ob mice fed the stock diet and the HF cafeteria diet, and slightly lower in ob/ob mice fed the HC cafeteria diet. The extent of hyperglycaemia and hyperinsulinaemia was not significantly different in the three groups of ob/ob mice, but plasma GIP concentrations were considerably raised (75%) in the group of ob/ob mice receiving the HF cafeteria diet.

Intestinal length and weight were characteristically greater in stock fed ob/ob mice than in lean mice (Fig. 2). The HF and HC cafeteria diets did not significantly alter intestinal length in ob/ob mice, but intestinal weight was lower in ob/ob mice receiving the HF cafeteria diet. The intestinal concentration and content of GIP were about 200% greater in stock fed ob/ob mice than in lean mice, and the HF cafeteria diet increased the intestinal GIP concentration (118%) and content (71%) compared with stock fed ob/ob mice. The intestinal GIP concentration and content were not significantly altered by the HC cafeteria diet, although the mean values were higher (45% and 25% respectively) than in stock fed ob/ob mice.

Quantitative evaluation of histological sections of upper jejunum confirmed that the density of K cells in this region of intestine is similar in lean and ob/ob mice fed a stock diet (Fig. 3). However, the density of K cells was increased in ob/ob mice receiving the HF (33% and 54%) and HC (25% and 45%) cafeteria diets compared with the lean and ob/ob mice fed the stock diet. The distribution of K cells was similar in all sections, being mainly at the neck of the crypts and near the base of the villi, with very few cells in the apical region of the villi. There were no apparent differences in the intensity of staining.
Intestinal length and weight, and intestinal concentration and content of gastric inhibitory polypeptide (GIP) in lean (+/+) mice fed stock diet, and in obese (ob/ob) mice fed either stock diet, a high fat (HF) cafeteria diet or a high carbohydrate (HC) cafeteria diet. Values are mean ± SEM of 6 mice. *P < 0.05 compared with lean mice fed stock diet. a: P < 0.05 compared with obese HF cafeteria diet; b: P < 0.05 compared with obese HC cafeteria diet; c: P < 0.05 compared with obese stock diet.

**Fig. 2.**

Discussion

Obese (ob/ob) mice readily consumed the varied and palatable cafeteria diets, showing a greater preference for the HF than the HC cafeteria foods (Bailey et al. 1985). Although energy intake from the HF cafeteria diet and the stock diet was similar, body weight was greater in mice receiving the former diet, supporting previous evidence that metabolic efficiency is increased by this diet (Bailey et al. 1985). Whereas the extent of basal hyperglycaemia and hyperinsulinaemia in ob/ob mice was not significantly changed by the cafeteria diets, plasma GIP concentrations were raised by the HF cafeteria diet. This cannot be attributed to hyperalimentation since ob/ob mice consumed similar amounts of the HF and stock diets, thus substantiating acute studies showing a potent GIP-releasing effect of fatty acids (Brown 1982; Flatt et al. 1984a). A sustained insulinotropic...
effect of GIP in ob/ob mice appears to be dependent upon a rise in the extent of hyperglycaemia (Flatt et al. 1984a), which may explain why the raised GIP concentrations in the HF cafeteria fed ob/ob mice were not associated with a further increase in basal hyperinsulinaemia.

Consistent with previous observations, the stock fed ob/ob mice exhibited an increased intestinal concentration and content of GIP compared with lean mice (Flatt et al. 1983). This was associated with a similar density of K cells in the upper jejunum, although increased numbers of K cells have been observed in more distal regions of the small intestine in older ob/ob mice (Polak et al. 1975). Feeding a HF cafeteria diet to ob/ob mice produced jejunal K cell hyperplasia with a further increase of the intestinal GIP concentration and content. Thus, chronic stimulation of GIP release by increased consumption of fats appears to promote K cell proliferation and function. K cell hyperplasia of the upper jejunum was also observed in ob/ob mice fed the HC cafeteria diet. A concomitant increase of the intestinal GIP concentration and content was not observed, suggesting that the GIP content of K cells in HC cafeteria fed ob/ob mice may be less than in HF cafeteria fed ob/ob mice. This coincides with the relative potencies of carbohydrate and fat for the stimulation of GIP release (Flatt et al. 1984a), but differences in the intensity of K cell staining in mice fed the cafeteria diets were not apparent with the present technique. The lack of a significant effect of the HC cafeteria diet (83% metabolisable energy from carbohydrate) on plasma and intestinal GIP compared with stock fed ob/ob mice may reflect the already high carbohydrate component of the stock diet (66% metabolisable energy from carbohydrate).

It has been suggested that GIP increases glucagon secretion in states of glucose intolerance (Salera et al. 1982). Thus GIP might contribute to the hyperglycaemia in ob/ob mice by maintaining inappropriately raised plasma glucagon concentrations (Flatt et al. 1982). GIP has also been reported to stimulate lipogenesis in adipose tissue (Brown 1982). Hence, in combination with the hyperinsulinaemia, raised GIP levels might promote obesity in ob/ob mice. The present study indicates that in addition to enhanced GIP secretion, dietary fat is also an important determinant of K cell number and intestinal GIP content in ob/ob mice.

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References


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