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Gastrointestinal Transit in Relation to Gut Endocrine Cells in Animal Models of Human Diabetes[1)]

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ABSTRACT

Gastrointestinal transit was measured in non-obese diabetic (NOD) mice, as an animal model of human diabetes type 1, and in obese diabetic mice, as an animal model of human diabetes type 2. The endocrine cells known to correlate to gastrointestinal transit, namely secretin, serotonin, Peptide YY (PYY) and enteroglucagon cells, were identified by immunocytochemistry and quantified by computer image analysis in different segments of the gut. Gastrointestinal transit was significantly accelerated in NOD mice and slower in obese diabetic mice than in controls. The density of duodenal secretin and serotonin as well as colonic PYY and enteroglucagon cells in NOD mice was significantly higher than that of control mice. On the other hand, the density of duodenal secretin and serotonin cells was significantly lower in obese diabetic mice than in controls. It was concluded that changes in duodenal secretin and colonic serotonin, PYY and enteroglucagon cells may play a role in accelerated gastrointestinal transit in NOD mice and delayed gastrointestinal transit in obese diabetic mice.

INTRODUCTION

The frequency of gastrointestinal complications in patients with diabetes varies in different studies between 25% and 76% (9,10,15,20,23). Symptoms such as nausea and vomiting, abdominal pain, diarrhoea, constipation and faecal incontinence are common in these patients (9,10,15,20,23). Gastrointestinal complications in patients with diabetes are attributed to disturbed gastrointestinal motility (1,12). The endocrine cells of the gut secret peptide and amines that play an important role in regulating gastrointestinal motility (2,3,19). Abnormality in some of these endocrine cells has been reported in animal models of human diabetes (6,7,8,21,22). It is not clear,

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however, whether or not these models have gastrointestinal motility disorders similar to patients with diabetes.

The aim of the present study was to establish whether there is a gastrointestinal transit abnormality in animal models of human diabetes, where abnormality in the gut endocrine cells has been reported (6,7,21,22). It was also intended to establish if this is accompanied by an abnormality in the density of gut endocrine cell types shown recently to correlate to motility disorders in diabetic patients, namely secretin, serotonin, peptide YY (PYY) and enteroglucagon cells (8).

MATERIALS AND METHODS

Animals

Female non-obese diabetic (NOD) mice aged 20 weeks (Bomholtgård Breeding and Research Centrum, Denmark) were used as an animal model of human diabetes type 1. As controls, age- and sex-matched NOD mouse sister strain (Fujishima et al., 1989), BLAB/cJ, were used. As an animal model of human diabetes type 2, 15-week-old male homozygous obese diabetic mice (ob/ob, Umeå/Bom-ob, Bomholtgård Breeding and Research Centrum) were used. Lean homozygous (+/+) mice (Bomholtgård Breeding and Research Centrum) were used as controls. The mice were kept in our vivarium, five in each cage, in a room illuminated for 12 h/day with artificial light. They were fed a standard pellet diet (R 34, Lactamin, Vadstena, Stockholm) and given water ad libitum. The urine of each mouse was tested daily with Ecur test strips (Boehringer Mannheim). An animal was considered diabetic when glucosuria exceeded 50 mmol/l and remained at that level. The NOD was treated by i.p. injection of bovine insulin-Zn in suspension, 40 IU/ml (LenteR Mc, Novo). The animals received 1 IU every second day. If an animal lost weight or appeared unwell, the blood glucose level was measured through a blood sample taken from the tail. In the case of hyperglycaemia, the insulin dose was increased up to 5 IU or injected with 1 ml glucose solution (25 mg/ml) i.p. in the case of hypoglycaemia. Glucosuria was measured before every treatment to evaluate the treatment regime. The obese diabetic mice did not receive any treatment. Ten NOD mice with diabetes for 28-35 days and 10 BLAB/cJ mice, as well as 10 obese diabetic mice with diabetes for a duration of 29 days and 10 lean controls, were used in this investigation.

The animals were weighed on the day of the experiment. After the gastrointestinal transit had been determined, the animals were anaesthetised with a mixture of 1 ml midazolam (Dormicum, Roche, Switzerland), 1 ml fentanyl/fluanson (hypnorm, Janssen, the Netherlands) and 2 ml distilled water. The mice were injected with 0.2-0.3 ml of this mixture intra-peritoneally. Blood samples were taken by heart punctu-

re, and tissue samples of the splenic part of the pancreas, antrum (representative of the stomach), proximal duodenum (representative of the small intestine) and distal colon (representative of the large intestine) were excised. The pancreas was frozen in liquid nitrogen and kept at -70°C for insulin radioimmunoassay. The other tissue samples were fixed overnight in 4% buffered formaldehyde, embedded in paraffin wax and cut at 5 mm. The local committee on animal ethics at Umeå University approved the investigation.

Characterisation of the animals

Blood glucose concentration was measured on the day of the experiment with a glucometer (Precision Q.I.D., Medisense, Waltham, MA, USA), using Sensor Electrode Plus (Medisense). Serum insulin level and pancreatic insulin content were determined by radioimmunoassay. The pancreatic tissue specimens were allowed to thaw and then weighed. Insulin was extracted by boiling the tissue in 3 ml 0.5 M acetic acid, followed by homogenisation and centrifugation for 20 min at 700X g. The supernatant was neutralised with 1 M NaOH and stored at -70°C until the time for the assay. Insulin was measured in pancreatic tissue extracts and serum using a standard rodent insulin kit from DiaSorin, Inc. (Stillwater, MN, USA). The assays were performed according to protocols supplied by the manufacturer, in duplicates of undiluted extracts and serum and of 1:2 diluted extracts.

Gastrointestinal transit

The gastrointestinal transit was measured according to the method described by Piallai and Bhargava (1984). In brief, after an overnight fast the animals were orally given 0.2 ml of a standard meal for 3 min. This consisted of 10% charcoal in 5% arabic gum aqueous suspension. After 20 min, the animals were killed, and the gastrointestinal tract was excised carefully without stretching. The distance travelled by the charcoal and the total length of the small intestine were then measured. Gastrointestinal transit was expressed as the proportion (%) of the distance travelled by the charcoal along the entire length of the small intestine.

Immunocytochemistry

The sections were immunostained by the avidin-biotin complex (ABC) method (Dakopatts A/S, Glostrup, Denmark), as described in detail previously (4). Briefly, the sections were incubated with the primary antibody overnight at room temperature. Incubation was carried out for 30 min at room temperature with biotinylated swine anti-rabbit IgG (Dakopatts) diluted 1:200. The sections were incubated with the avidin-biotin-peroxidase complex (Dakopatts) diluted 1:100 for 30 min at room temperature. They were then immersed in 50 ml Tris-buffer; pH 7.4, containing 10

ml of 30% H2O2 and 25 mg diaminobenzidine tetrahydrochloride (DAB) followed by slight counter-staining in Mayer's haemotoxylin. The primary antiserums used were: rabbit anti-porcine secretin (Eurodiagnostica, Malmö, Sweden, code no. R787502 B33-1, dilution 1:1600), rabbit anti-serotonin (Eurodiagnostica, code no. R871204 B56-1, dilution 1:1600), rabbit anti-bovine peptide YY (PYY) (Eurodiagnostica, code no. R841303 B-4, dilution 1:1600) and rabbit anti-porcine pancreatic glucagons (Eurodiagnostica, code no. R781101, dilution 1: 1000, specific for N-terminus and cross-reacts with pancreatic glucagon and enteroglucagon).

Specificity controls were the same as those described previously (4). They included replacing the primary antibodies with non-immune rabbit serum and pre-incubation of the antibodies for 24 h at 4°C with the corresponding or structurally related antigen (75 µg/ml diluted antibody).

Computeised image analysis

The Quantimet 500MC image processing and analysis system (Leica, Cambridge, UK) linked to an Olympus microscope type BX50 was used. The software used in this system was Leica's Windows-based image analysis tool kit "QWIN" (version 1.02) and an interactive programming system, QUIPS (version 1.02). When quantification was performed in this system with an X20 objective, each pixel corresponded to 0.414 µm, and each field seen in the monitor represented 0.04-mm2 area of the tissue. The numbers of various endocrine cell types were measured as described previously using an automatic standard sequence analysis operation (5). Briefly, the number of immunostained cells was counted using field measurements. The total area of the epithelial cells was measured using a threshold setting. The data from each field was tabulated, computed and statistically analysed automatically. Measurements were taken in 40 randomly chosen fields (20 from the crypts and 20 from the villi) in the duodenum and in 20 fields in the antrum and colon for each peptide/amine and animal. These fields were chosen from three to five sections, at least 50 µm apart.

Statistical analysis

Comparisons between diabetic mice and controls were made with the Wilcoxon non-parametric test. P-values below 0.05 were considered significant.

RESULTS

Characterisation of the animals

The characteristics of the animals used are summarised in Table 1. While the body weight of diabetic NOD mice was significantly lower than that of controls, the body

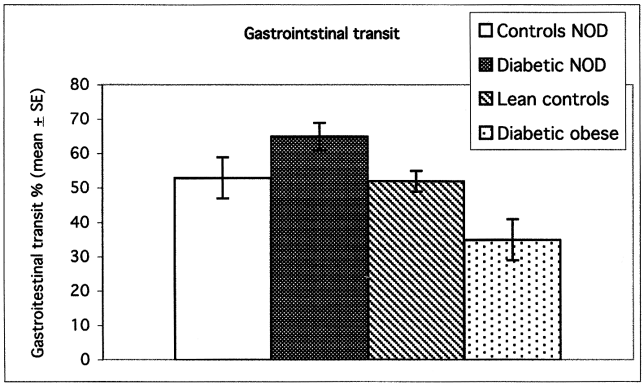
weight of diabetic obese mice was higher. There was no statistical significant difference between the diabetic NOD mice and controls regarding the concentrations of blood glucose and serum insulin. The blood glucose level and serum insulin concentrations were higher in obese diabetic mice than in controls. Whereas the concentration of pancreatic insulin was lower in diabetic NOD than in controls, it was higher in obese diabetic mice than in controls.

Gastrointestinal transit

Gastrointestinal transit in diabetic NOD mice and obese diabetic mice as well as in controls is illustrated in Fig.1. The gastrointestinal transit was significantly faster in diabetic NOD mice and slower in obese diabetic mice than in controls (P = 0.03 and 0.02, respectively).

Table 1. Characteristics of the animals used (all values expressed as mean+SE)

	Body weight (g)	**Blood glucose (mmol/l)**	**Serum insulin (pg/ml)**	**Pancreatic insulin (ng/g)**
NOD controls	27±0.5	5.9±0.2	33±14.4	2000±265
NOD diabetic	23±0.5***	6.8±1.9	26±9	0±0***
Lean controls	34±0.9	6.6±0.6	33±14.4	2184±302
Obese diabetic	53±1.3***	17.4±1.6***	70±21.5*	35127±6879***



*= P<0.5
**= P<0.01
***= P<0.001.

Fig. 1. Gastric emptying in non-obese diabetic (NOD) and obese diabetic mice as well as controls. *P<0.05.

Immunocytochemistry

In the antrum, serotonin-immunoreactive (IR) cells were found in both diabetic NOD mice and obese diabetic mice as well as in control mice. The endocrine cells were localised mostly in the glandular tissue. The endocrine cells were round to shape. In the duodenum, secretin- and serotonin-IR cells were observed in both dia-

betic mice and controls. Whereas serotonin-IR cells were found both in villi and crypts, secretin-IR cells were confined almost exclusively to the villus epithelium (Figs. 2 and 3). The shape of these cells varied from flask-shaped with a thin luminal process to a shape with a basal process running parallel to the basement membrane. In the colon, PYY-, enteroglucagon-, and serotonin-IR cells were found in both diabetic and control mice. The endocrine cells occurred mostly in the middle part of the colonic crypts. Their shape varied from basket-shaped to flask-shaped (Fig. 4).

Specificity controls showed that the immunostaining was abolished completely after the pre-incubation with the corresponding antigen. Pre-incubation of the antisera with the structurally related peptides had no effect on the immunostaining. Replacing the antisera with non-immune rabbit serum gave no staining.

Computer image analysis

The results of the morphometeric measurements are given in Figs. 5, 6 and 7. Whereas the density of duodenal secretin- and serotonin-IR were higher in NOD mice than in controls, they were lower than controls in obese diabetic mice. While the density of colonic PYY and enteroglucagon cells was significantly higher in diabetic NOD mice, the density of these cells in obese diabetic mice did not differ from that of controls. There was no statistical significant difference between diabetic and control mice regarding the colonic serotonin cells.

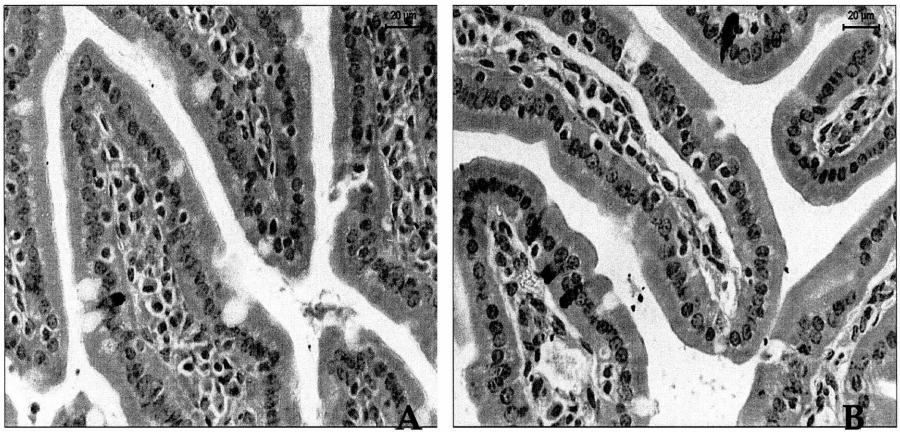


Fig. 2. Secretin-immunoreactive cells in the duodenum of a control (A) and of a NOD mouse.

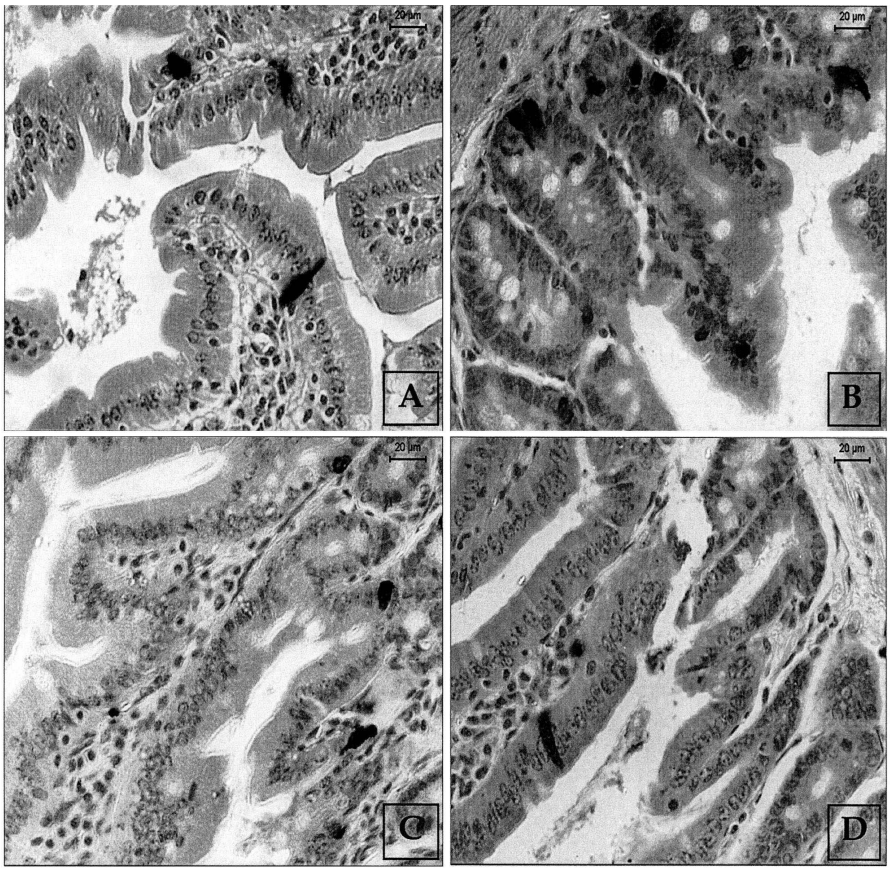


Fig. 3. Duodenal serotonin-immunoreactive cells in a control mouse (A), in a NOD mouse (B), in a lean control mouse (C) and in an obese diabetic mouse.

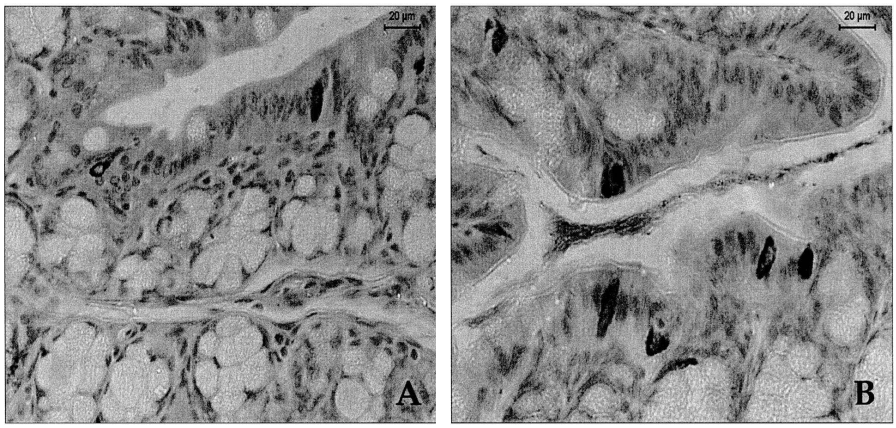


Fig. 4. Colonic peptide YY (PYY)-immunoreactive cells in a control lean mouse (A) and in an obese diabetic mouse (B).

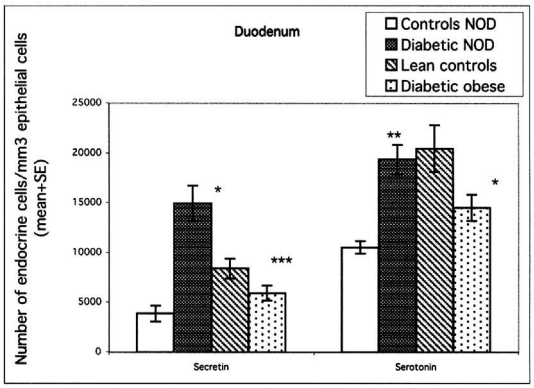


Fig. 5. The density of antral serotonin cells in NOD and obese diabetic mice as well as in controls.

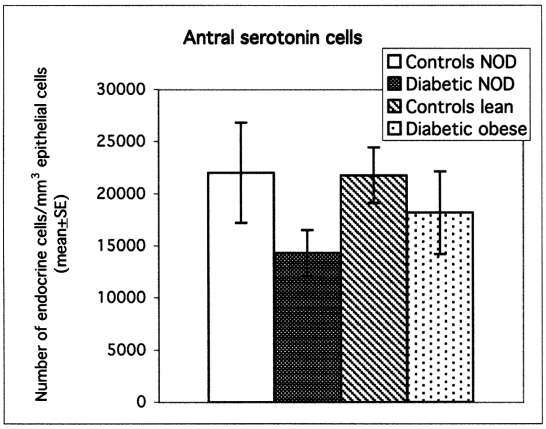


Fig. 6. The density of secretin- and serotonin-immunoreactive cells in the duodenum of NOD and diabetic mice as well as of controls. *P<0.05; **P<0.01; ***P<0.001.

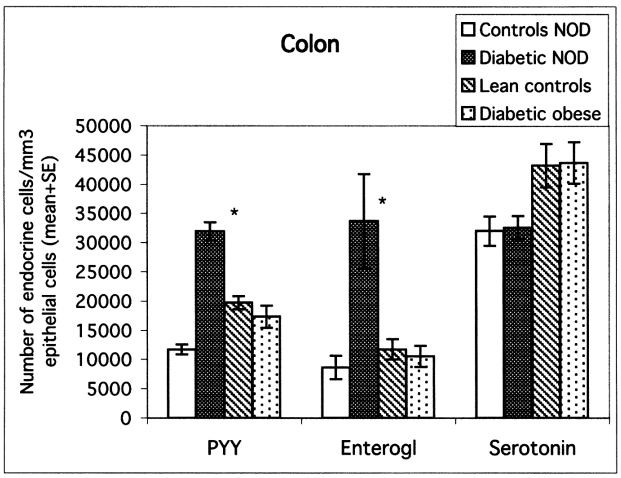


Fig. 7. Colonic PYY-, enteroglucagon- and serotonin-immunoreactive cells densities in NOD and obese diabetic mice as well as in controls. PYY = peptide YY; Enterogl = enteroglucagon; *P<0.05.

DISCUSSION

The present study showed disturbed gastrointestinal transit in animal models with spontaneously developed diabetes similar to human diabetes types 1 and 2. The nature of the disturbance differed, however, between the animal model of human diabetes type 1 and the animal model of human diabetes type 2. The animal model of human diabetes type 1 showed a fast gastrointestinal transit, whereas the animal model of human diabetes type 2 exhibited a slow gastrointestinal transit. Both animal models showed an abnormal endocrine cell density. The abnormality in the density of endocrine cell types studied differed, however, in the two animal models. Thus, whereas the densities of duodenal secretin- and serotonin-IR cells were higher in NOD mice, they were lower in obese diabetic mice. Furthermore, the densities of colonic PYY- and enteroglucagon-IR were higher in NOD mice, but were not altered in the obese diabetic mice.

The present observation of a high density of duodenal secretin-IR cells in NOD mice is in agreement with previous findings, where numerous secretin cells have been found (7). The present finding of a low density of duodenal secretin-IR cells in obese diabetic mice disagrees with our previous observation that the density of these cells was unaltered (22). In the previous study, the same immunocytochemical method and antibodies, as well as the same morphometeric approach as in the present study were used. Whereas the animals used in the previous investigation were studied immediately after the onset of diabetes, the animals used in the present study had a longer duration of diabetes. One may speculate that the density of duodenal secretin-IR cells in obese diabetic mice is normal at the onset of diabetes, but tends to decrease with the increased duration of the diabetic state. Secretin is known to delay gastric emptying (13). The high density of duodenal secretin cells in NOD mice and the density of these cells in obese diabetic mice may be secondary to the fast and slow gastrointestinal transit that occurred in NOD and obese diabetic mice, respectively.

The present findings of a high density of duodenal serotonin-IR cells in NOD mice disagree with our previous result that the density of these cells was low in diabetic NOD mice (7). The animals investigated here had, however, a longer duration of diabetes than those investigated previously. The difference could be explained on the basis of the changes brought about by the diabetic state. On the other hand, the low density of duodenal serotonin-IR cells in obese diabetic mice observed here is in agreement with our previously published report (22). Serotonin has been found to stimulate gastric antrum, small intestinal and colonic motility, as well as accelerated gastric emptying, and both small and large intestinal transit (14,24,25). The high density of duodenal serotonin-IR cells in NOD mice and the low density of these cells in obese diabetic mice may be one of the factors responsible for the accelerated

gastrointestinal transit observed in NOD mice and the slow gastrointestinal transit that occurred in obese diabetic mice.

In the present study, the density of colonic PYY-IR cells was high in NOD mice with a long duration of diabetes. Previously, The density of these cells has been found to be low in those animals with an immediate onset of diabetes (21). The present observation of an unaltered density of PYY-IR cells in the colon of obese diabetic mice agrees with earlier findings in these animals (22). PYY is one of the mediators of ileal brake; it delays intestinal transit and gastric emptying (16). One may speculate that the increase in colonic PYY-IR cell density observed here in diabetic NOD mice may be a secondary response to accelerated gastrointestinal transit seen in these animals.

The present observation of a high density of enteroglucagon cells in the colon of NOD mice with a long duration of diabetes differs from that found in patients with a long duration of diabetes type 1, where the density of enteroglucagon cells were low (8). This difference may due to NOD mice possibly not being a model that resembles human diabetes type 1 in all aspects.

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