Modulation of intestinal brush border membrane chemical composition during postnatal development in rats: Effect of gestational diabetes

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There was a significant increase in fucose (52%), total hexoses (16%) and hexosamine (56%) except sialic acid, which was reduced (77%) in the microvillus membrane of infants born to rat mothers made diabetic by injecting alloxan on day 3 of gestation. Expressed on the protein basis there were a significant increase in membrane, triglyceride, total cholesterol, and phospholipids content of brush border in pups from diabetic group between 5-45 days of postnatal age. Intestinal morphology in diabetic group showed, regression of tubular glands, distorted cellular organization of mucosal cells, reduction in the mucosal cell height and number of secretory goblet cells. These findings suggest that the gestational diabetes affects the sugar and lipid composition of the intestinal brush border membrane in rats during early stages of the postnatal development, which may be associated with compromised tissue functions later in life.

Keywords: Brush border membrane composition, Gestational diabetes, Postnatal development, Small intestine.

The growth and development of the mammalian foetus is primarily determined by its genetic make-up and nutrients supplied by the mother during gestation. However, genetically predetermined growth and development can be influenced by intrauterine environment in which the development of foetus occurs¹. There are critical, specific and restricted periods during development, coincident with periods of rapid cell proliferation, during which, tissues and organs differentiate and mature in preparation for survival after birth. Either a stimulus or insult during such a critical period may have long lasting consequences, on the developing tissues and organs postnatally². Disturbed maternal metabolism or inappropriate maternal food intake impairs the nutrient supply to the developing foetus and confronts the foetus with an unfavorable intrauterine milieu which affects the development of various foetal tissues and organs post parturition³,⁴.

Gestational diabetes (GD) is one such metabolic disorder, which confronts the foetus with an abundant glucose supply and alters the intrauterine environment of the developing foetus. It is characterized by an enhanced placental transport of glucose and other nutrients to the foetus, which results in foetal and neonatal macrosomia⁵,⁶. Approximately 7% of all pregnancies are complicated by GD, resulting in more than 2,00,000 cases annually. Changes in enzyme and glucose transport in intestine of pups born to diabetic rat mothers have been reported⁷. However, whether the observed changes are related to chemical composition of intestinal brush borders is not known. Since intestinal tissue is specifically responsible for digestion and absorption of nutrients, critical to postnatal growth, any aberration in its composition would be expected to affect growth of offsprings. Thus, the effect of GD on chemical composition of brush border membranes during postnatal development of small intestine has been studied using rat as the experimental model, since it closely reflects the early events in human diabetic pregnancies.

Materials and Methods

Animals—Female Wistar strain albino rats weighing 125-150 g were procured from the Central Animal House of Panjab University, Chandigarh, India. They were housed in propylene cages and maintained at 22° ± 3°C, on a 12:12 h light dark cycle and a minimum 40% RH. Standard pellet diet (Ashirwad Industries, India) and water were given ad libitum. The animals were acclimatized to the laboratory conditions for 1 week before initiating the
experiments. The experimental protocol was approved by the ethical committee of the Institute on the use of laboratory animals. Experiments on animals were performed in accordance with the guidelines for the use of laboratory animals.

**Experimental protocol**—Females rats were caged overnight with males and the presence of spermatozoa in the vaginal smear was taken as an indicator day 0 of pregnancy. Pregnant rats were selected and housed individually. Pregnant female rats were randomly divided into two groups (control and diabetic) of 6 each. Rats were made diabetic by injecting (120 mg/kg body weight, ip) alloxan (Central Drug House, Mumbai, India) on day 3 of pregnancy. Induction of diabetes was confirmed by determining glucose levels in the blood taken from the tail vein. Animals in the control group were injected with saline. Pups in the control and the diabetic groups were kept with their mothers for 3 weeks post-parturition. Thereafter, animals were given standard rat pellet diet ad libitum with free access to water until they were sacrificed.

**Preparation of intestinal microvillus membranes**—Body weight of pups born was recorded on alternate days and animals were sacrificed at 5, 10, 14, 21, 30, and 45 days of postnatal age under light ether anesthesia. Intestine starting from ligament of Treitz to caecum was removed and washed thoroughly with ice-cold isotonic NaCl solution. Intestinal weight and length were measured. The brush border membranes were isolated and purified by the method of Kesseler *et al.*

Briefly, intestinal tissue was homogenized in ice-cold buffer containing 50 mmol/L mannitol and 2 mmol/L Tris (pH 7.2). Solid CaCl$_2$ was added to the homogenate (final concentration, 10 mmol/L) and allowed to stand on ice for 15 min followed by centrifugation at 3000 g for 15 min at 4°C. The supernatant was re-centrifuged at 42,000 g for 30 min at 4°C. The pellet obtained was suspended in 50 mmol/L sodium maleate buffer, pH 6.8. The membrane preparation exhibited 12 to 13 fold enrichment of brush border marker enzymes, lactase (suckling animals), and sucrase (adult animals) over the crude homogenate.

**Biochemical estimations**—Membrane sugars, sialic acid, fucose, hexose, hexosamine and membrane lipids were extracted by the method of Folch *et al.*

Triglyceride, total cholesterol, phospholipids were quantified using standard methods respectively. Protein was estimated by the method of Lowry *et al.* using bovine serum albumin as the standard.

**Intestinal morphology**—Histopathological sections were prepared by the method of Bradbury taking full thickness sections from the jejunal segments of different groups of rats. After fixation in buffered 10% formaldehyde, tissues were embedded in paraffin, solid sections were cut at 5 µm thickness and stained with haematoxylin and eosin. The sections were examined under light microscope and photographs were taken.

**Statistical analysis**—The statistical analysis of the data was done using paired Student’s *t*-test. *P*-value changes <0.05 were considered significant.

**Results**

The blood glucose level was significantly elevated (20%) in rat mothers of diabetic group. A state of sustained hyperglycaemia prevailed till parturition. The blood glucose level of pups born to diabetic mothers was significantly high (92-22%) compared to control (Table 1). The hyperglycaemia was observed up to 45 days after birth under the experimental conditions. The effect of gestational diabetes on various biochemical parameters is presented in Fig. 1. Membranes prepared from infants of diabetic animals showed an increase in cholesterol/phospholipids molar ratio at day 5 (24%) and at day 21 (22%) of postnatal age compared to control (Table 2). There was essentially no difference in the ratios in control and diabetic groups at day 30-45 of postnatal age.

Intestinal tissue in 10 day old pups, born to diabetic rat mothers, showed irregularity in structural organization of different cellular components as compared to control (Fig. 2). The mucosal lining showed thinning and distorted cellular organization (2b). The mucosal folds in the intestine of the 21 day old pups born to control mothers showed normal development of secretory folds possessing columnar

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<th>Table 1—Blood glucose levels of pups born to control and diabetic rat mothers</th>
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<td>[Values are mean ± SD from 4-6 observations]</td>
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<tr>
<td>Postnatal age (days)</td>
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*P* values; * <0.001, ** <0.01, *** <0.05
Fig. 1—Effect of gestational diabetes on sialic acid (a), fucose (b), total hexoses (c), hexosamine (d), triglyceride (e), total cholesterol (f) and phospholipids (g) contents of MVM in developing rat intestine. [Values are mean ± SD from 4 animals each. P: * <0.001, ** <0.01, *** <0.05.]
cells and appearance of goblet cells (2c). In contrast, pups born to diabetic mothers at this stage revealed diminished mucosal and columnar cell height along with a decrease in the number of secretory goblet cells (2d).

**Discussion**

Rat model closely reflects the early events in human diabetic pregnancies. Hence, drug-induced diabetes in rodents provides a useful tool to study developmental abnormalities associated with diabetes.

In the present study, the effect of gestational diabetes on the chemical composition of rat intestinal brush border membrane was investigated. Hyperglycaemic condition during early postnatal stage may have severe impact on the structure and function of developing cells/tissues. Gestational diabetes modulates the postnatal development of brush border enzymes and transport functions in rat intestine under sustained hyperglycaemic conditions. Postnatal development of intestine exhibit marked alterations in the chemical composition of microvillus membrane (MVM) lining the luminal surface of enterocytes. Cholesterol/phospholipids molar ratio in MVM of rat intestine during postnatal development (Table 2). Jacobs demonstrated alterations in the brush border protein glycosylation under the influence of prolonged exposure to glucose in diabetes in adult animals. MVM on the luminal surface of intestine is rich in glycoproteins and glycolipids. Chu and Walter reported, that activities of sialyl and fucosyl transferase are reciprocally related during postnatal development in the rat small intestine. A decline in sialyl transferase activity and an increase in fucosyl transferase activity occurred concurrently during weaning period. Therefore, the observed changes in the sialic acid and fucose content of MVM could

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<th>Control</th>
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<td>45</td>
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Table 2—Cholesterol/phospholipids molar ratio in MVM of rat intestine during postnatal development [Values are mean of two determinations from pooled fractions of the membranes from 4 animals]

Fig. 2—Morphology of small intestine in pups born to control and diabetic rat mothers at 10 (a and c) and 21(b and d) days of postnatal age. Arrows indicate the morphological changes, as explained in the text. [Magnification = 400X].
prematurely be attributed to the effect of maternal diabetes on the activities of sialyl and fucosyl transferase enzymes in the developing rat intestine. In addition the availability of sugar nucleotides and acceptor molecules may also influence the membrane glycosylation under these conditions.

Altered levels of the different membrane lipids found in the current study are in agreement with earlier studies. Schwarz et al. reported that MVM in suckling animals contained greater amounts of total lipids, cholesterol and phospholipid per milligram protein than mature MVM preparations. A similar decrease in cholesterol and phospholipid content with age was demonstrated by Engehardt et al. Decrease in phospholipid was more than cholesterol which resulted in an increase in cholesterol/phospholipid molar ratio upon maturation. Gestational diabetes does not affect the pattern of alteration in lipid composition of MVM during postnatal development. A number of studies in adult rats have shown that diabetes mellitus induces alterations in the lipid composition of MVM. Chemical composition of brush borders in diabetic rats revealed elevated levels of cholesterol, triglycerides and cholesterol/phospholipid ratios. Feingold et al. demonstrated enhanced cholesterol synthesis in epithelial cells of small intestine of streptozotocin-induced diabetic rats. Alterations in lipid fluidity and lipid composition in MVM of enterocytes prepared from chronic streptozotocin-induced diabetic rats has also been reported. The relatively low fluidity of these membranes compared to controls appear to be due to higher cholesterol/phospholipid ratio in the membranes of diabetic animals, which resulted from higher cholesterol content with no change in phospholipid content in diabetic membranes. Dutt and Sarkar reported a significant increase in the lipid contents in intestinal BBM of diabetic rats, suggesting observed changes to be responsible for altered functions of the diabetic intestine. Thus gestational diabetes induces modifications in the chemical make-up of MVM lining enterocytes in pups during postnatal development. This may suggest that diabetes in general affect the chemical structure of brush border membrane in intestine.

The light microscopy showed thinning and distorted cellular organization of mucosal cell lining in 10 day old intestine of pups born to diabetic mother. Intestinal tissue from 21 and 45 day old animals from diabetic group showed reduction of mucosal cell height along with decrease in the number of secretory goblet cells and regression of tubular glands. These findings are in agreement to those of Reusens-Billen et al., who reported growth retardation of the intestinal mucosa in pups of severely diabetic mothers. Though the time sequence in the appearance of differentiated cells was identical in the gut mucosa of control and diabetic groups, a general hypotrophy of the intestine has been reported. Morphometric evaluation of the foetal pancreatic β-cells in the rats indicates that maternal diabetes in the rat retard the development of the foetal β-cells. The volume density and the weight of the β-cells were considerably low in infants of diabetic mothers than in control counterparts. Amri et al. have demonstrated that exposure to hyperglycaemia in utero can cause a nephron deficit, which may have renal consequences later in life. Enamel hypoplasia has been reported in the foetus of the rats with alloxan-induced diabetes mellitus. The frequency of enamel hypoplasia was dependent on the severity of the hyperglycaemia in the mothers. Abnormalities in the foetal lungs include whole lung hypoplasia and hypotrophy has been reported by Bourbon et al. under the influence of gestational diabetes. These findings suggest that a general impaired growth of various organs with morphological changes in the offspring of diabetic mothers during postnatal development is a characteristic of gestational diabetes. The role of endocrine imbalances under these conditions however cannot be ruled out in this process.

Conclusion
The present findings indicate that gestational diabetes affects the sugar and lipid composition of the microvillus membrane during postnatal development in rat intestine, which may be associated with compromised tissue function as reported previously.

Acknowledgement
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