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Morpho-mechanical intestinal remodeling in type 2 diabetic GK rats—Is it related to advanced glycation end product formation?

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ABSTRACT

Little is known about the mechanisms for the biomechanical remodeling in diabetes. The histomorphology, passive biomechanical properties and expression of advanced glycation end product (N epsilon-(carboxymethyl) lysine, AGE) and its receptor (RAGE) were studied in jejunal segments from 8 GK diabetic rats (GK group) and 10 age-matched normal rats (Normal group). The mechanical test was done by using a ramp distension of fluid into the jejunal segments *in vitro*. Circumferential stress and strain were computed from the length, diameter and pressure data and from the zero-stress state geometry. AGE and RAGE were detected by immunohistochemistry staining. Linear regression analysis was done to study association between the glucose level and AGE/RAGE expression with the histomorphometric and biomechanical parameters. The blood glucose level, the jejunal weight per length, wall thickness, wall area and layer thickness significantly increased in the GK group compared with the Normal group ($P<0.05$, $P<0.01$ and $P<0.001$). The opening angle and absolute values of residual strain decreased whereas the circumferential stiffness of the jejunal wall increased in the GK group ($P<0.05$ and $P<0.01$). Furthermore, stronger AGE expression in the villi and crypt and RAGE expression in the villi were found in the GK group ($P<0.05$ and $P<0.01$). Most histomorphometric and biomechanical changes were associated with blood glucose level and AGE/RAGE expression. In conclusion, histomorphometric and biomechanical remodeling occurred in type 2 diabetic GK rats. The increasing blood glucose level and the increased AGE/RAGE expression were associated with the remodeling, indicating a causal relationship.

1. Introduction

It is now widely recognized that gastrointestinal (GI) disorders are common in diabetic patients (Horowitz and Samsom, 2004). Diabetes can affect the entire GI tract including the small intestine. Similar to other complications of diabetes, the duration of the disease and poor glycemic control are associated with the severity of the GI problems (Horowitz and Samsom, 2004). However, the pathogenesis of GI disorders in diabetes mellitus is complex in nature, multi-factorial (motor dysfunction, autonomic neuropathy, glycemic control, psychological factors, etc.) and is not well understood (Horowitz and Samsom, 2004). Our previous studies have shown that the morphological and biomechanical properties of the GI tract were changed in type-1 diabetic patients (Frokjaer et al., 2007) and in diabetic animals (Yang et al., 2004; Zhao et al., 2003, 2007). Such remodeling was also demonstrated in the esophagus and stomach from type-2 diabetic animals (Liao et al., 2006; Zhao et al., 2009). However, little is known about the mechanisms for the biomechanical remodeling during diabetes.

Advanced glycation end products (AGEs) are formed physiologically and they gradually increase with aging. The formation is accelerated in diabetes (Méndez et al., 2010). AGEs can lead to structural and functional changes directly in the target protein. They also can bind to their receptor (RAGE) leading to activation of signaling pathways to induce a series of changes (Ramasamy et al., 2011). AGEs and RAGE have been demonstrated to play an important role in diabetic complications, e.g. cardiovascular complication (Jandeleit-Dahm and Cooper, 2008; Barlovic et al., 2010), retinopathy (Zong et al., 2011) and nephropathy (Yamamoto et al., 2007; Busch et al., 2010). AGEs and RAGE are also associated with arterial wall stiffening in diabetes (Sims et al., 1996; Wolffenbuttel et al., 1998). Therefore, AGE and RAGE may also play an important role for diabetic complications of the GI tract. We have demonstrated that AGE and RAGE were up-regulated in small intestine and colon in STZ-induced diabetic

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rats (Chen et al., 2012). However, until now data on the effect of AGEs and RAGE on the diabetic GI tract are sparse. To the best of our knowledge, data on the distribution of AGE and RAGE in the intestine of type-2 diabetes have not been reported.

The Goto-Kakizaki (GK) rat is a model of non-insulin-dependent diabetes mellitus: type 2 diabetes (Goto and Kakizaki, 1981). It exhibits similar metabolic and hormonal disorders as human diabetes disease (Goto et al., 1988; Miyamoto et al., 1996). One study has demonstrated small intestine hyperplasia and increased total activity of disccharidases in the GK rat model (Adachi et al., 2003). However, data related to the histomorphometric and biomechanical properties of the small intestine have not been reported so far. The aims for this study were to study the histomorphometric and biomechanical properties of jejunal segments in the GK rats. The AGE and RAGE expression in the jejunal segments were also studied. Furthermore, the association between blood glucose and AGE/RAGE with histomorphometric and biomechanical parameters were analyzed.

2. Materials and methods

Eight inherited type 2 diabetic rats (Goto-Kakizaki rats, GK group), 12 weeks old and weighting about 330 g, were purchased from Taconic Europe DK-8680 Ry, Denmark. Ten age-matched normal rats (same strain as the GK rats) served as control (Normal group). During the breeding, the body weight was measured every week from 12 weeks. The rats drank tap water and ate food without restrictions. The fasting glucose level of the rats was measured once every second week starting from 16 weeks and also at the last day of the experiment. The rats survived for 32 weeks and were fasting overnight before the experiments. Approval of the protocol was obtained from the Danish Committee for Animal Experimentation.

2.1. Experimental procedures

The experimental procedures were similar to those reported by us before (Zhao et al., 2003, 2006). Briefly, the rats were anaesthetized with Hypnorm 0.5 mg and Dormicum 0.25 mg per 100 g body weight (Hypnorm: Dormicum: sterile water=1:1:2; subcutaneous injection). The proximal part of the jejunal segment from 5 cm distal to the ligament of Treitz was harvested after opening the abdomen. The residual contents in the lumen were gently cleared using physiological saline and the wet weight was measured. A 2-cm-long tissue was cut from each end of the segments and fixed in 10% formalin for histological and immunohistochemistry examination. Then two rings from the proximal end of the segment were cut and used for no-load state and zero-stress state analysis. The segment (7 cm-long) was put into the organ bath and immersed in the Krebs solution of the following composition (mmol/L): NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; NaH_2PO4, 1.0; MgCl, 1.2; ascorbic acid, 0.11 aerated with a gas mixture of 95% O_2–5% CO_2 with 0.25% EGTA at pH 7.4. Two cannulas were fixed on the two sides of organ bath. The proximal and distal ends of each intestinal segment were tied on the two cannulas on two sides in the organ bath with silk threads. The proximal end connected via a tube to a fluid container containing Krebs solution for applying luminal flow (rate 0.66 ml/min) by pump. The distal end was closed. The segment was inflated with Krebs solution using a ramp distension protocol from 0 to 10 cm $\cdot H_2O$ three times. The outer diameter was videotaped by a CCD camera (Sony, Japan) through a stereomicroscope and the lumen pressure was measured simultaneously.

For obtaining data on the zero-stress state, the rings were collected as mentioned above and placed in the Krebs solution. A photograph was taken of the cross-section of the rings in the no-load state. Then each ring was cut radially when immersed in the fluid under the microscope. Each ring opened up into a sector. Photographs of the zero-stress state were taken ~60 min after the radial cutting to allow viscoelastic creep to take place.

2.2. The measurements of outer diameter

The circumferential diameter of inflating tissue was measured by analyzing the video clip. In order to analyze the videos automatically, several imaging techniques were employed such as simple color thresholding for tissue area segmentation, morphological operation for de-noising and third order polynomial curve fitting of the tissue shape for determining the circumferential direction. The analysis software has developed in the C++ language with Microsoft Direct Show SDK library for movie handling.

2.3. Mechanical data analysis

The morphometric data were obtained from digitized images of the segments in the zero-stress and no-load states. Measurements were undertaken using image analysis software (Sigmascan ver. 4.0, Sigma Corp., San Rafael, CA, USA). The following data were measured from each specimen: the circumferential length (C), the wall thickness (h), the wall area (A), and the opening angle at zero-stress state (α). Furthermore, the outer diameter (D) and the length (L_p) were measured from the images of the pressurized segments as mentioned above. The subscripts i, o, n, z and p refer to the inner (mucosal) surface, outer (serosal) surface, no-load state, zero-stress state and pressurized condition. The opening angle α was defined as the angle subtended by two radii drawn from the midpoint of the inner wall to the inner tips of two ends of the specimen.

The measured data was used for computation of biomechanical parameters defined as:

Residual Green's strain at the mucosal surface:

$$E_i = \frac{(C_{i-n}/C_{i-z})^2 - 1}{2} \tag{1}$$

Residual Green's strain at the serosal surface:

$$E_o = \frac{(C_{o-n}/C_{o-z})^2 - 1}{2} \tag{2}$$

The stress and strain of the jejunal segment in the pressurized state were determined under assumptions that the wall was homogenous and the intestinal shape was cylindrical. Series calculation was done as the methods reported before (Zhao et al., 2002) and the different parameters in the pressurized state, such as longitudinal stretch ratio λ_ϕ, the luminal radius r_{i-p}, the outer radius r_{o-p}, the wall thickness $h_p$and the circumferential stretch ratio λ_θ were obtained. Then the Kirchhoff's stress and Green's strain at a given pressure were computed according to the following equations:

Circumferential Kirchhoff's stress:

$$S_\theta = \frac{\Delta \Pr_{i-p}}{h_p \lambda_\theta^2} \tag{3}$$

Longitudinal Kirchhoff's stress:

$$S_\phi = \frac{\Delta \Pr_{i-p}^2}{h_p \lambda_\phi^2 (r_{o-p} + r_{i-p})} \tag{4}$$

Circumferential mid-wall Green's strain:

$$E_\theta = \frac{\lambda_\theta^2 - 1}{2} \tag{5}$$

Longitudinal Green's strain:

$$E_\phi = \frac{\lambda_\phi^2 - 1}{2} \tag{6}$$

ΔP is the transmural pressure difference. The longitudinal mid-wall stretch ratio was referenced to the no-load state because tissue strips could not be cut for obtaining the zero-stress state in longitudinal direction. However, the longitudinal mid-wall length in rat intestine does not differ between the no-load and zero-stress states (Dou et al., 2006).

The stress–strain curve was fitted using the exponential function equation.

$$S = (S^* + b)e^{a(E-E^*)} - b \tag{7}$$

where S is the stress, E is the strain, and S^* and E^* are the stress and the strain at an arbitrary point on the stress–strain curve. The constants a and b were obtained from the exponential function.

2.4. General histological staining

After the samples fixed in 10% phosphate-buffered formalin over for 24 h, they were dehydrated in a series of graded ethanol (70%, 96% and 99%) and embedded in paraffin. Five-micron sections were cut perpendicular to the mucosa surface and the paraffin was cleared from the slides with coconut oil (over 15 min. 60 °C). The sections were rehydrated in 99%, 96% and 70% ethanol followed by a 10 min wash in water and stained with Haematoxylin and Eosin (HE). The layer thickness was measured by the same pathologist in a blinded review and 16 determinations were made on each specimen and averaged.

2.5. Immunohistochemical staining

2.5.1. Tissue pretreatment

The tissue samples for immunohistochemistry were also fixed in 10% phosphate-buffered formalin for 24 h and embedded in paraffin. Five-micron sections were cut perpendicular to the mucosa surface and placed in a water bath at 40 °C. Thereafter, sections were transferred onto pretreated microscopic slides