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• BASIC RESEARCH •

Morphologic and biomechanical changes of rat oesophagus in experimental diabetes

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Abstract

AIM: To study morphologic and biomechanical changes of oesophagus in diabetes rats.

METHODS: Diabetes was induced by a single injection of streptozotocin (STZ). The type of diabetes mellitus induced by parenteral STZ administration in rats was insulin-dependent (type I). The samples were excised and studied *in vitro* using a self-developed biomaterial test machine.

RESULTS: The body mass was decreased after 4 d with STZ treatment. The length of esophagus shortened after 4, 7, 14 d. The opening angle increased after 14 d. The shear, longitudinal and circumferential stiffness were obviously raised after 28 d of STZ treatment.

CONCLUSION: The changes of passive biomechanical properties reflect intra-structural alteration of tissue to a certain extent. This alteration will lead to some dysfunction of movement. For example, tension of esophageal wall will change due to some obstructive disease.

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INTRODUCTION

Esophagus is a distensible muscular tube that connects pharynx and stomach. The function of the esophagus is to transport food by peristaltic movement, which is the result of the interaction of the tissue forces in the esophageal wall and the hydrodynamic forces in the food bolus. Esophagus has been studied by radiography[1], concurrent videofluoroscopy and manometry[2,3], high-frequency ultrasonography[4-6], and endoscopic sclerotherapy[7,8]. Motility disorders[9], bolus transport[10,11], systemic sclerosis[12], pain[13], wall distensibility[8], impedance planimetric characterization[14] and the effects of epidermal growth factor[15] on esophagus have been reported in many papers. Since the function of esophagus is mainly mechanical, our work was focused on providing quantitative measurement of passive biomechanical properties of esophagus. Many investigations on biomechanics of esophagus are available in the literature[16,17]. Gregersen *et al*. studied strain distribution in the layered wall[18,19], relation between pressure and cross-sectional area[20] and other biomechanical properties[21-23] of esophagus. A more recent work used a novel ultrasound technique to study the biomechanics of the human esophagus *in vivo*[24]. Patel represented biomechanical and sensory parameters of the human esophagus at four levels[25]. Researchers have done a lot biomechanical studies on gastrointestinal tract such as intestine[26,27], small intestine[28-32], ileum[33], duodenum[34] and large intestine[35,36].

Most previous studies have explained the relationship between the diabetes and gastrointestinal tract function[37,38]. Some researches studied relationship between esophageal dysfunction and neuropathy[39], oesophagus scintigraphy[40] and the relationship between esophageal motility and transit[41] in diabetic patients. More recently, Jorgensen reported tension-strain relations and morphometry of rat small intestine in experimental diabetes[42]. Zhao introduced the remodeling of zero-stress state of small intestine in streptozotocin-induced diabetic rats[43].

This paper presents the effect of experimental diabetes on the morphologic and biomechanical properties of the esophagus. The result of this study indicated that experimental type I diabetes caused significant changes in the passive biomechanical properties in the rat esophagus.

MATERIALS AND METHODS

Materials

Diabetes was induced by a single injection of streptozotocin (STZ). The form of diabetes mellitus induced by parenteral STZ administration in rats is insulin-dependent (type I). Twenty-seven rats were divided into 4 groups according to the survival time after STZ treatment: 4 d (n = 7), 7 d (n = 7), 14 d (n = 7), 28 d (n = 6). Another 8 rats were used as normal controls. The samples were taken from the middle part of esophagus. Two rings were cut from each end of the sample to measure the geometric parameters of the no-load state and the opening angle at zero-stress state. The remaining part was excised and studied *in vitro* using a self-developed biomaterial test machine.

Methods

Using this machine, the esophagus was stepwise elongated and inflated and continuously twisted in circumferential-longitudinal direction. In the normal controls and 28 d of diabetes group, after the intact esophagus was tested, the mucosa and muscle layers were separated using microsurgery and tested in the same loading procedure as mentioned above. The esophagus was treated as a membrane when the stress and strain were calculated, the longitudinal and circumferential stresses were considered to be evenly distributed along the wall thickness while the radial stress and other transverse shear stresses were

ignored. The torque *vs* twist-angle relation was approximately linear at a specified pressure and longitudinal stretch ratio. Thus, the shear modulus can be computed by the torque, twist angle and polar moment of inertial at this state. However, the shear modulus varied greatly with the changing inflation pressure and longitudinal stretch ratio.

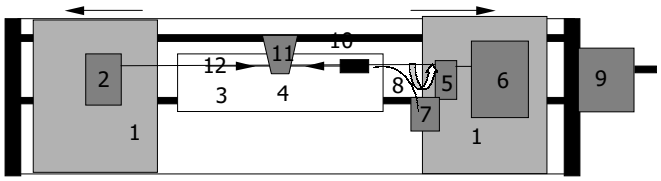

Figure 1 Simplified diagram of biomaterial test machine.1: Linear stage, 2: Torque transducer, 3: Organ bath, 4: Specimen, 5: Force transducer, 6: Motor for axial rotation, 7: Pressure transducer, 8: Infusion channel, 9: Motor for linear stage, 10: Rails for linear stage, 11: CCD camera, 12: Plastic rod.

RESULTS

Type I diabetes could induce the following effect on the biomechanical and morphologic properties of esophagus: body weight and morphology, shear modulus, circumferential and longitudinal stress-strain relationship, stress-strain relationship of muscle layer and mucosa layer.

Body weight and morphology

The body mass kept a steady increase in the control rats. But it went down after 4 d in the diabetes rat (Figure 2A). The length of esophagus *in vivo* obviously declined after 4, 7, 14 d, but it would return to normal level after 28 d (Figure 2B). The mass per unit lengh *in vitro* changed little (Figure 2C). In the intact esophagus, the opening angle increased after 14 d of STZ treatment (Figure 2D).

Shear modulus

Changes of elastic shear moduli in the course of diabetes development at longitudinal stretch ratio λ_{zz} = 1.5 and various transmural pressure are shown in Figure 3A. Elastic shear modulus would rise with increased transmural pressure. Especially when transmural pressure was more than 0.25 kPa, the shear moduli for various transmural pressure were remarkably different. And diabetes has notably affected the shear modulus. This effect showed that shear moduli are obviously increased after 28 d.

Changes of elastic shear modulus in the course of diabetes development at transmural pressure P = 1 kPa and various longitudinal stretch ratio are pictured in Figure 3B. Elastic shear modulus would rise with increased longitudinal stretch ratio. Shear moduli were remarkably different at various longitudinal stretch ratios. And diabetes has notably affected the shear modulus. This effect demonstrated that shear moduli were obviously increased after 28 d of STZ treatment.

Circumferential and longitudinal stress-strain relationship

Figure 4A shows the changes of circumferential stress-strain relationship in the course of diabetes development at longitudinal stretch ratio λ_{zz} = 1.5 and various transmural pressure. All curves of experimental group inclined to left side except that after 4 d. The curve after 28 d was on the most left side. The circumferential stiffness increased after 7, 14, 28 d of diabetes.

The changes of longitudinal stress-strain relationship in the course of diabetes development at transmural pressure P = 0.25 kPa and various longitudinal stretch ratio are pictured in Figure 4B. The stress-strain curve after 28 d was obviously inclined to left side. So the longitudinal stiffness notably increased after 28 d.

Stress-strain relationship of muscle layer and mucosa layer

The circumferential stress-strain relationship of muscle layer and mucosa layer in the process of inflation at a longitudinal stretch ratio of 1.5 is pictured in Figure 5A. And the experimental diabetes was after 28 d. For muscle layer, there was no obvious difference between the control and diabetes groups. For mucosa layer, the stress-strain curve moved to left side in parallel. So circumferential stiffness of mucosa layer with diabetes was larger than that of control.

Figure 5B shows longitudinal stress-strain relationship of muscle layer and mucosa layer in the process of elongation at a transmural pressure of 0.25 kPa. For muscle layer, there was no obvious difference between control and diabetes groups. There was no notable difference for mucosa layer either.

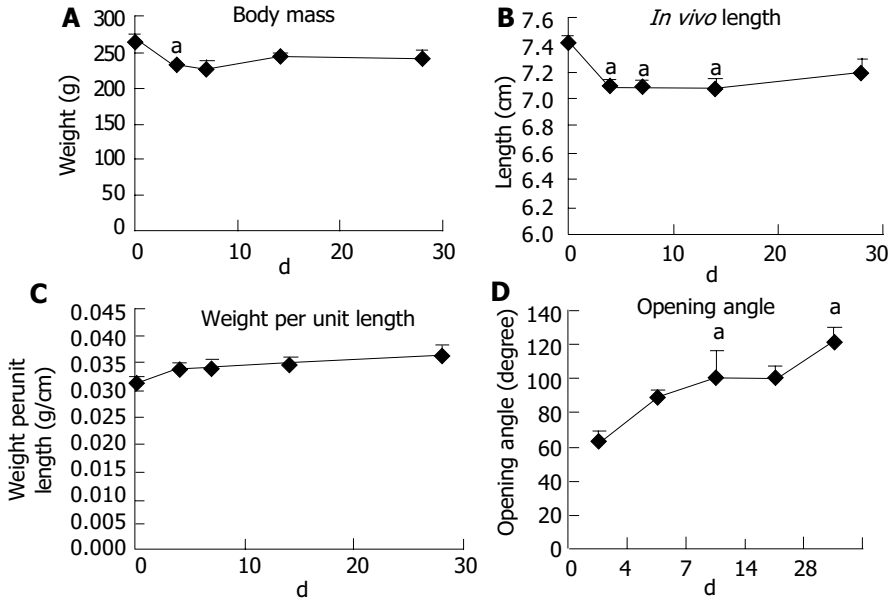

Figure 2 Changes of body mass and esophagus morphology and opening angle at zero-stress state in the process of diabetes development. Dunner's test result: significant difference *vs* normal control (^aP<0.05). A: Change of body mass, B: Change of *in vivo* length, C: Change of mass per unit length, D: Change of opening angle.

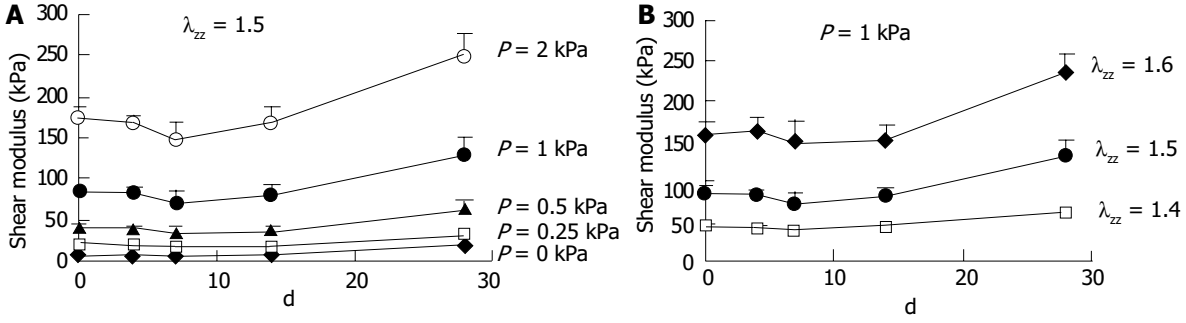

Figure 3 Change of elastic shear modulus in the process of diabetes development. A: Change at λ_{zz} = 1.5 and various transmural pressure, B: Change at P = 1 kPa and various longitudinal stretch ratio.

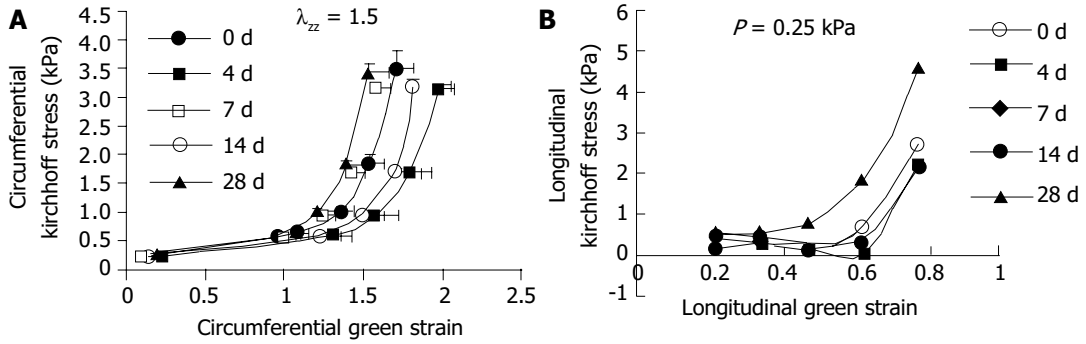

Figure 4 A: Change of circumferential stress-strain relation in the course of diabetes development at λ_{zz} = 1.5 and various transmural pressure. B: Change of longitudinal stress-strain relation in the course of diabetes development at P = 0.25 kPa and various longitudinal stretch ratio.

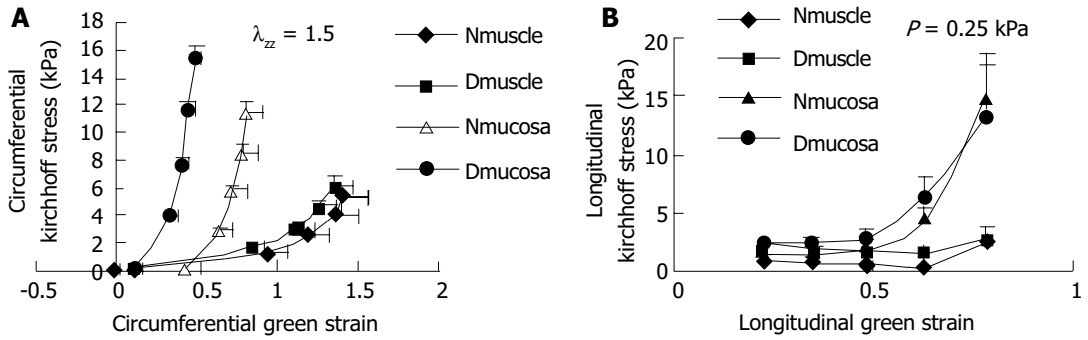

Figure 5 A: Circumferential stress-strain relation between muscle layer and mucosa layer in the process of inflation at a longitudinal stretch ratio of 1.5. N: Normal control, D: 28 d of diabetes. B: Longitudinal stress-strain relation between muscle layer and mucosa layer in the process of elongation at a transmural pressure of 0.25 kPa. N: Normal control, D: 28 d of diabetes.

DISCUSSION

A large number of studies have discovered that diabetes can affect the movement of oesophagus. Transportation of oesophagus may delay or slow down, and movement of esophagus can not coordinate.

This dysfunction of movement can be a result of muscle and nerve cooperative failure[39-41,44-47]. Histologic research has proved that diabetes can destroy vagus nerve[48]. Though there are many papers on movement and function of oesophagus in diabetes, few data on morphologic and passive biomechanical properties are seen. The change of passive biomechanical properties reflects intra-structural alteration of tissue to a certain extent. This alteration will result in some dysfunction of movement, for example, tension of esophageal wall will change due to some obstructive disease[49,50], and therefore, it is necessary to study biomechanics and morphology together.

The body mass is decreased in rat with diabetes. This is consistent with other studies[43,51]. Diabetes will lead to hyperplasia of some organs. Hyperplasia of esophagus is less frequent than that of small intestine[52,53]. Diabetes has caused rise of the opening angle of small intestine[44], also it is seen for esophagus.

In this paper, the shear, longitudinal and circumferential stiffnesses were obviously elevated after 28 d with STZ treatment. Jorrensen[42], Liu[54] and Zhao[51] have discovered that stiffness is raised in diabetes in small intestine, blood vessel and arterial wall.

We can draw a conclusion that the changes of passive biomechanical properties reflect intra-structural alteration of tissue to a certain extent. This alteration will lead to some dysfunction of movement.

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