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Involvement of Rictor/mTORC2/Akt/GLUT4 pathway in the regulation of energy metabolism in the gastric smooth muscle of diabetic rats

Sun Yan[1#], Yu-Rong Zheng[2#], Zheng Jin[1], Mo-Han Zhang[1]✉ and Xiang-Shu Cui[2]✉

[1]Yanbian University College of Medicine, Yanji 133000, Jilin, China; [2]Yanbian University School of Nursing, Yanji 133000, Jilin, China

Diabetes mellitus can be accompanied by a variety of complications. The purpose of the present study was to characterize the Rictor/mTOR complex 2 (mTORC2)/Akt/glucose transporter 4 (GLUT4) pathway and its effects on energy metabolism in the gastric smooth muscle of diabetic rats. Diabetes was induced in rats using streptozotocin and their phenotype was compared with untreated rats. The relationship between gastric motility and energy metabolism was analyzed by comparing the contraction and ATP metabolism of muscle strips. Western blotting was used to detect the expression of key proteins in the pathway. The diabetic rats demonstrated less frequent and less powerful gastric smooth muscle contractions. The concentrations of ADP, AMP, and ATP, and the energy charge in gastric smooth muscle changed in different periods of diabetes, and these changes were consistent with changes in mechanistic target of rapamycin (mTOR) protein content. The expression of the key intermediates in signal transduction in the Rictor/mTORC2/Akt/GLUT4 pathway also underwent significant changes. Rictor protein expression increased during the development of diabetes, but the activation of mTORC2 did not increase with the increase in Rictor expression. GLUT4 translocation is regulated by Akt and its expression change during the development of diabetes. These findings suggest that altered energy metabolism is present in gastric smooth muscle that is associated with changes in the Rictor/mTORC2/Akt/GLUT4 pathway. Rictor/mTORC2/Akt/GLUT4 pathway may be involved in the regulation of energy metabolism in the gastric smooth muscle of diabetic rats and the development of diabetic gastroparesis.

Keywords: Gastric smooth muscle; Rictor; mTORC2; Akt; Glucose uptake

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✉e-mail: mhzhang@ybu.edu.cn (M-HZ); xscui@ybu.edu.cn (X-SC)
#Sun Yan and Yu-Rong Zheng contributed equally to this work, should be considered co-first authors.
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INTRODUCTION

Diabetic gastroparesis is a gastric motility disorder characterized by delayed gastric emptying. The underlying reduction in gastric motility is directly related to changes in energy metabolism in gastric smooth muscle cells. ATP is the universal energy currency in cells and is predominantly generated in mitochondria by the action of ATP synthase (Kimoloi, 2018), although glucose can also be metabolized *via* the glycolytic pathway in the cytoplasm to generate a small amount of ATP.

Extensive research over the past two decades has established a central role for mechanistic target of rapamycin (mTOR) in the regulation of many fundamental cell processes, from protein synthesis to autophagy, and deregulated mTOR signaling is implicated in the progression of cancer and diabetes, as well as in the aging process (Kleinert *et al.*, 2014). Since mTOR is a protein kinase related to energy metabolism and diabetes, the putative relationship between mTOR and diabetes should be further explored. mTOR is a serine/threonine protein kinase and a member of the phosphoinositide 3-kinase (PI3K)-related kinase (PIKK) family. It forms the catalytic subunit of two structurally and functionally distinct protein complexes: mTOR complex 1 (mTORC1) and complex 2 (mTORC2) (Kleinert *et al.*, 2014; Saxton & Sabatini, 2017). Each complex has its own unique roles but they also interact with each other. mTORC1 is a major regulator of protein synthesis and cell growth, and comprises three components: mTOR, regulatory protein associated with mTOR (Raptor), and mammalian lethal with Sec13 protein 8 (GβL; mLST8) (Kimoloi 2018; Hara *et al.*, 2002). Recently, it has been shown that mTORC1 senses glucose through more than one mechanism (Kimoloi 2018; Kleinert *et al.*, 2014; Zhang *et al.*, 2017), and it also responds to intracellular and environmental stresses that are incompatible with growth, such as low ATP concentration. A reduction in cellular energy during glucose deprivation activates the stress-responsive metabolic regulator AMP-activated protein kinase (AMPK), which inhibits mTORC1 both indirectly, through the phosphorylation and activation of tuberous sclerosis complex 2 (TSC2), and directly, through the phosphorylation of Raptor (Kimoloi, 2018; Hara *et al.*, 2002; Zhang *et al.*, 2017). Most studies have pointed out that mTORC1 is involved in energy metabolism, but mTORC2 is rarely studied.

Rictor is essential for the integrity and stability of the mTORC2 complex (Kimoloi, 2018; Knudsen *et al.*, 2020). Low Rictor expression leads to lower SIN1 expression and *vice versa* (Hara *et al.*, 2002; Knudsen *et al.*, 2020). Studies have found that the deletion of Rictor gene affects the body's absorption of glucose. Diabetes mellitus is the disorder of glucose absorption and utilization. This suggests that Rictor may play a role in diabetes pathogenesis. It has been reported that alterations to the mTORC1 signaling pathway in skeletal muscle directly affects whole-body energy homeostasis (Mukaida *et al.*, 2017). mTORC2 is also a major upstream kinase for the Akt Ser473 residue (Kleinert *et al.*, 2014; Mu-

kaida *et al*., 2017). The activation of Akt promotes glucose transporter 4 (GLUT4) translocation to the plasma membrane, which leads to increases in glucose uptake, glucose metabolism, and ATP synthesis (Mukaida *et al*., 2017; Cosimo *et al*., 2019; Hasannejad *et al*., 2019). At the same time, the interaction between mTORC1 and mTORC2 may also affect the energy metabolism of the body.

Glucose is an important source of energy for muscle contraction. Furthermore, normal glucose metabolism is essential for the maintenance of good health, and abnormal glucose metabolism can lead to serious health problems, such as diabetes. Increases in glucose uptake are due to translocation of glucose transporters from intracellular storage vesicles in adipocytes and skeletal muscle cells to the plasma membrane (Khan & Kamal, 2019). GLUT4 is the principal transporter of glucose in muscle and fat cells and has a key role in the control of cellular glucose metabolism and whole-body energy homeostasis, which are strongly linked to type 2 diabetes (Zhao *et al*., 2019).

The purpose of the present study was to characterize the Rictor/mTORC2/Akt/GLUT4 pathway and its effects on energy metabolism in the gastric smooth muscle of diabetic rats.

MATERIALS AND METHODS

Animals

Forty 8-week-old Sprague-Dawley male rats, weighing 200 ± 20 g, were housed in single cages at 20–25°C, under a 12-h light/dark cycle, and had free access to food and water. All animal experimental procedures were approved by the Ethics Committee of Yanbian University College of Medicine.

Preparation of the animal model

A 0.5% solution of streptozotocin (STZ) (Cat No. S0130, SIGMA, USA) was prepared in 0.1 mmol/L citrate buffer (pH 4.0). 40 rats were fasted for 12 hours, after which 30 of them were randomly selected, weighed, and given a single intraperitoneal injection of STZ at 65 mg/kg to induce diabetes. The remaining 10 rats were intraperitoneally injected with the same dose of citrate buffer and comprised a normal control (NC) group. One week later, glucose levels were measured in blood taken from the tail vein, and rats with glucose concentration of ≥16.9 mmol/L were regarded as diabetic. One diabetic rat died following the STZ injection; therefore, one rat was randomly selected from the control group and injected with STZ. Thus, 30 diabetic rats and 9 normal rats were studied. The diabetic rats were randomly allocated to 4-week (DM4w), 6-week (DM6w), and 8-week (DM8w) endpoints (n=10 rats per group). The NC rats were allocated to three groups, with n=3 rats per group, and they were sacrificed at 4, 6, and 8 weeks.

Preparation of muscle strips and measurement of spontaneous muscular contraction

The rats were euthanized at the determined time points. For each individual, the entire stomach was dissected, had its contents removed, and its muscle tissue peeled off. Gastric antrum ring muscle strips were dissected and placed vertically in oxygen-saturated Krebs's buffer (NaCl 118 mmol/L, KCl 4.75 mmol/L, $CaCl_2$ 2.54 mmol/L, KH_2PO_4 1.2 mmol/L, $MgSO_4$ 1.2 mmol/L, $NaHCO_3$ 25 mmol/L, and glucose 10 mmol/L, Kermel, TianJing, China) and connected with a tension sensor and a RM-6240 multi-channel physiological signal recorder (SCCHENGYI, ChengDu, China). Before each experiment readout, a load of 1 g was applied to each muscle strip for 30 min. After the spontaneous contractions of the smooth muscle strips stabilized, the number of muscle contractions every 400 s was recorded as the frequency, and the height of the peaks was recorded as the amplitude of the muscle contractions. All experiments were repeated independently 8 times.

High-performance liquid chromatography (HPLC)

One hundred milligrams of fresh gastric antrum annular muscle were added at 10 g/mg/mL to 0.4 mol/L perchloric acid solution that had been pre-cooled to 4°C, and the muscle was homogenized in an ice-bath. The supernatant was obtained by centrifugation (10 000 rpm, 10 min) and its pH was adjusted to 6.5. Twenty microliter samples in triplicates at 4°C and at dilutions of 200, 100, 50, 20, and 10 mg/L were then subjected to HPLC to measure the concentrations of ATP, AMP, and ADP. A C18 column (4.6 mm×150 mm, 5 m; Shimadzu, Japan), a column temperature of 25°C, and a mobile phase of 100 mmol/L phosphate buffer (12 mmol/L disodium phosphate and 88 mmol/L sodium dihydrogen phosphate, pH=6.5) and methanol (99:1 volume ratio) were used. The flow rate was 1.0 mL/min and the UV detection wavelength was 254 nm. The energy charge was calculated according to the following formula: energy charge= (ATP+1/2ADP)/(ATP+ADP+AMP) (Wilbring *et al*., 2013). All experiments were repeated 8 times, independently.

Western blotting

Western blotting was used to detect protein levels of Akt, phospho-Akt Ser473, mTOR, phospho-mTOR Ser2481, and Rictor. The circular muscle of the gastric antrum was added to a precooled lysis buffer at 75–125 μl/g, homogenized, and incubated at 4°C for 30 min. The lysates were then centrifuged at 14 000 rpm for 30 min, the supernatants were obtained, and their protein concentration was determined. Samples containing 60 μg protein were subjected to SDS-PAGE, then electrotransferred to PVDF membranes. The membranes were then incubated with anti-Akt (1:1 000, Cat No. 9272, Cell Signaling Technology®, Inc., MA, USA), phospho-Akt (1:1 000, Cat No. 4060, Cell Signaling Technology®, Inc., MA, USA), mTOR (1:1 000, Cat No. 2792, Cell Signaling Technology®, Inc., MA, USA), phospho-mTOR Ser 2481 (1:500, SAB4301526, Sigma, MO, USA), Rictor (1:1,000, Cat No. 2974, Cell Signaling Technology®, Inc., MA, USA), or β-actin (1:500, A5316, Sigma, MO, USA) at 4°C overnight. After washing, the membranes were incubated with secondary antibody (1:1 000, Cat No. 7055, Cell Signaling Technology®, Inc., MA, USA) solution, washed, and exposed to a chromogenic agent. The expression of the proteins of interest was calculated relative to β-actin levels (A5316, Sigma, MO, USA).

Western blotting was also used to measure the relative expression of GLUT4 protein (rabbit anti-GLUT4 antibody, Cat No. ab654, Abcam, Cambridge, UK) in gastric antrum teres muscle. The tissue was lysed as described above. The supernatant (containing cytoplasmic proteins) was added to the cell membrane lysate and the samples were mixed and centrifuged. Next, the supernatant (containing cell membrane proteins) was collected and its

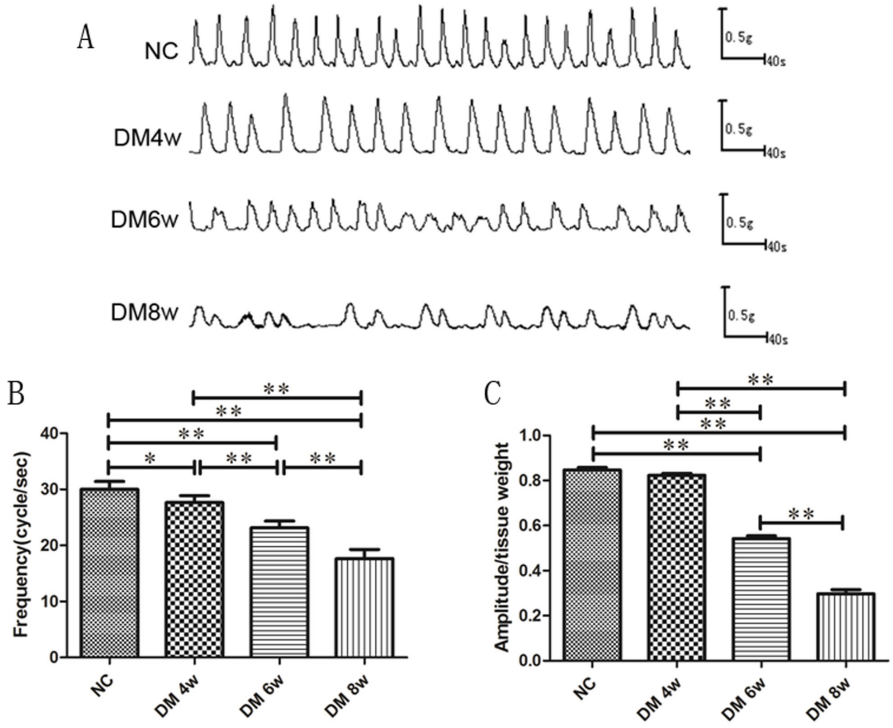

Figure 1. Amplitude of spontaneous contractions in normal gastric smooth muscle and at each time point during the progression of diabetes mellitus.
(**A**) Gastric smooth muscle strip motility analysis; (**B**) The frequency of spontaneous contraction of gastric smooth muscle. (**C**) The amplitude of the contractions of the gastric smooth muscle. The data are displayed as mean ± S.D.; *P<0.05, **P<0.01.

protein concentration was determined. Samples containing 60 μg of protein were subjected to SDS-PAGE and electrotransfer, as described above. The membranes were incubated with the antibody against GLUT4 (1:1,000) at 4°C overnight, or with or β-actin at (temperature, time), a secondary antibody (1:1 000), and a chromogenic agent, and the expression of GLUT4 was calculated relative to that of β-actin. All experiments were repeated 8 times, independently.

Statistical analysis

Statistical analyses were performed with SPSS 19.0 software, and the figures were made with GraphPad Prism5 software (GraphPad Software, San Diego, CA). All experiments were repeated at least 8 times. The data are expressed as mean ± standard deviation (S.D.). Differences between groups were compared using t-test and two-way analysis of variance (ANOVA). P<0.05 was considered to indicate a significant difference, P<0.01 was considered to indicate a highly significant difference.

RESULTS

Contraction frequency and amplitude in gastric smooth muscle

We evaluated the rate and amplitude of the contraction of stomach muscle strips from diabetic and normal rats (Fig. 1A). The number of muscle contractions during 400 s was recorded as the frequency and the height of each registered peak was recorded as the amplitude of muscle contraction. The frequency of spontaneous contractions of gastric smooth muscle was lower in DM4w, DM6w, and DM8w groups compared to the NC group (P<0.05 or P<0.01). This decrease in contraction frequency corresponded to the duration of diabetes: it was the lowest in the DM8w group (P<0.01 compared to DM6w, Fig. 1B). and both in the DM6w and DM8w groups displayed lower frequency than DM4w group (both P<0.01). The amplitude of the contractions of the gastric smooth muscle was decreased in the DM6w and DM8w groups compared to the NC group (both P<0.01), and compared to the DM4w group as well (both P<0.01). The amplitude of the contractions of the gastric smooth muscle was lower in the DM8w group than that in the DM6w group (P<0.01, Fig. 1C). Thus, the motility of rat gastric smooth muscle decreased with the progression of diabetes in the rats.

The concentrations of ATP, ADP, AMP and energy charge in gastric smooth muscle

ATP is the basic molecular unit of energy in the body; therefore, the amount of ATP present in a tissue and the rate of ATP synthesis (ADP/ATP) reflect overall energy metabolism in the tissue. We measured the concentrations of ATP, ADP, and AMP in the gastric smooth muscle of rats using HPLC (Fig. 2) and found that the concentration of ATP was not affected by diabetes (P>0.05). However, ADP concentration was decreased in the DM4w group (P<0.01), and increased in the DM6w and DM8w groups (both P<0.01) as compared to NC. It was also higher in the DM6w and DM8w groups than in the DM4w group (both P<0.01), and higher in the DM8w group than in the DM6w group (P<0.01, Fig. 2A). Therefore, a trend was observed for the ATP production (ADP/ATP ratio). The ADP/ATP ratio was the lowest in the DM4w group (P<0.05 compared to the NC) and the highest in the DM8w group (P<0.01 compared to the NC). The ADP/ATP ratio was higher

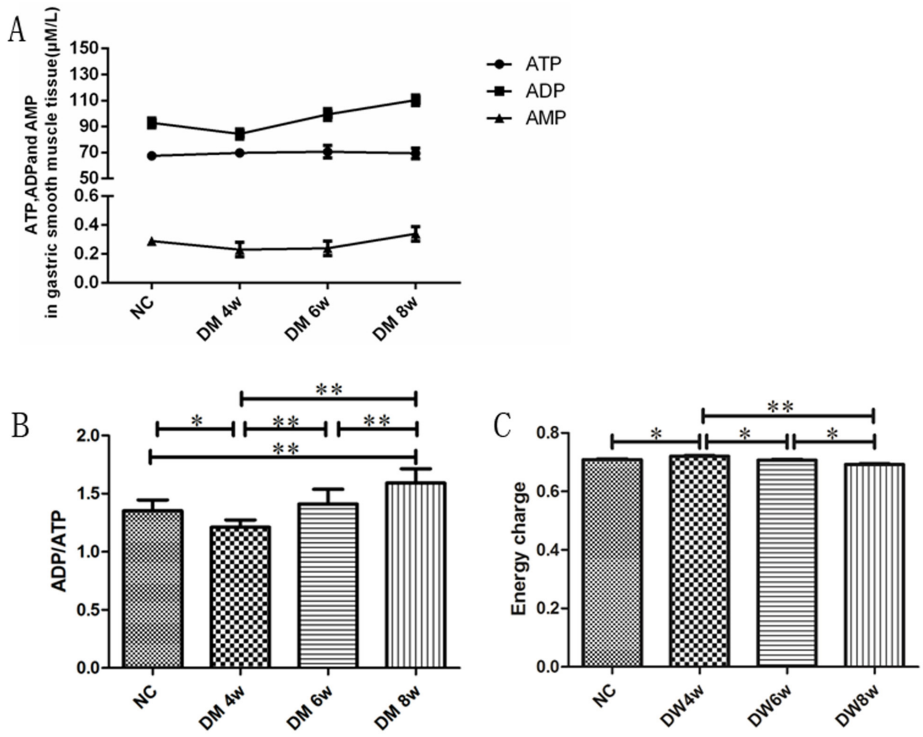

Figure 2. Concentrations of ATP, ADP, and AMP (μmol/L) in the gastric smooth muscle of diabetic rats.
(**A**) ATP, ADP, and AMP concentrations in control and diabetic rats. (**B**) ADP/ATP ratio in control and diabetic rats. (**C**) The energy charge in control and diabetic rats. The data are displayed as mean ± S.D.; **P*<0.05, ***P*<0.01.

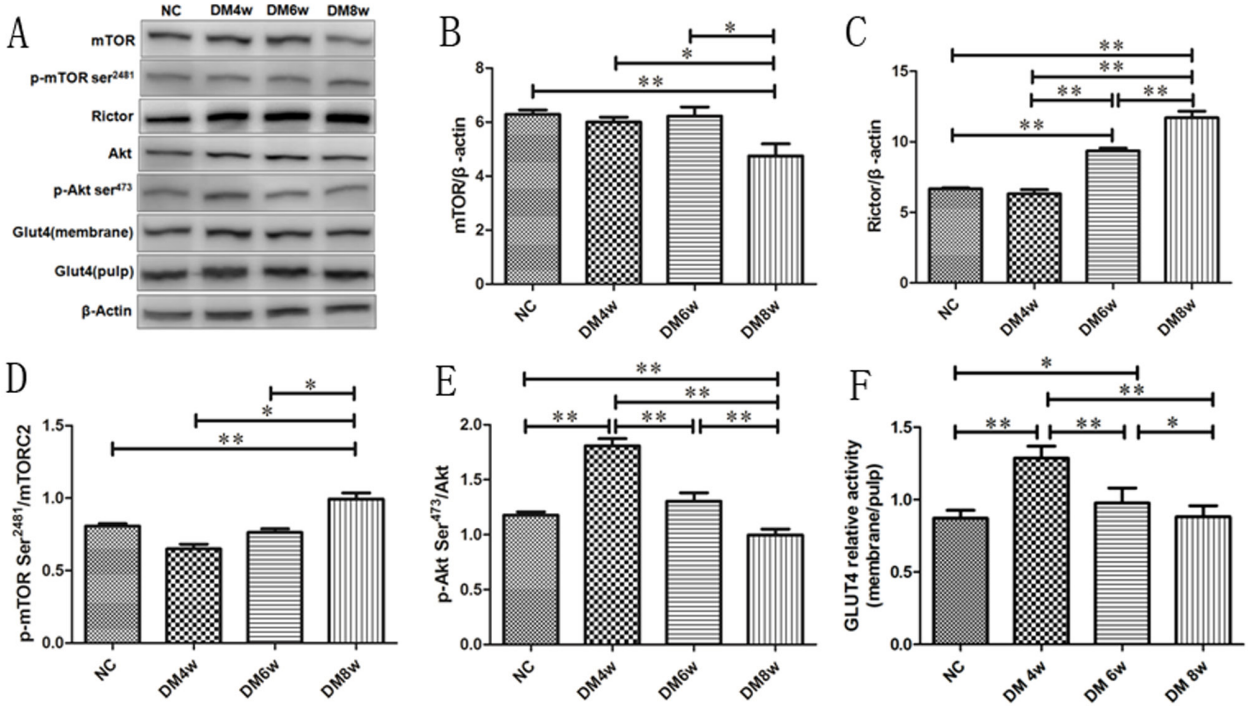

Figure 3. Protein expression of the key proteins in the Rictor/mTORC2/Akt/GLUT4 pathway in control and diabetic rats.
(**A**) Western blot of the key proteins in the Rictor/mTORC2/Akt/GLUT4 pathway in the rat gastric smooth muscle; (**B**) mTOR expression in the rat gastric smooth muscle; (**C**) Rictor expression in the rat gastric smooth muscle; (**D**) p-mTOR Ser2481/ mTOR ratio; (**E**) p-Akt Ser473/ Akt ratio; (**F**) GLUT4 expression in the gastric smooth muscle of control and diabetic rats. The data are displayed as mean ± S.D.; **P*<0.05, ***P*<0.01.

in the DM6w and DM8w than in DM4w (DM8w vs DM4w $P<0.01$, DM6w *vs* DM4w $P<0.01$,), and higher in DM8w than in DM6w ($P<0.01$, Fig. 2B). The energy charge of the gastric smooth muscle was increased in DM4w as compared to NC($P<0.05$). Conversely, the energy charge was decreased in the DM6w and DM8w-compared to DM4w ($P<0.05$, $P<0.01$), and was lower in DM8w than in DM6w ($P<0.05$, Fig. 2C). These results suggested that energy metabolism disorder was present in the gastric smooth muscle of diabetic rats, leading to the impairment of the energy supply to muscle and the development of diabetic gastroparesis.

Akt, phospho-Akt Ser473, mTOR, phospho-mTOR Ser2481, GLUT4, and Rictor protein expression in gastric smooth muscle

We used Western blotting to measure the expression of the key proteins in the Rictor/mTORC2/Akt/GLUT4 pathway the in gastric smooth muscle of rats in each group (Fig. 3A). mTOR expression in the gastric smooth muscle was decreased in the DM8w group compared to NC ($P<0.01$), DM4w ($P<0.05$), and DM6w ($P<0.05$) groups (Fig. 3B). Rictor is an important component of TORC2 but does not promote the phosphorylation of mTOR at Ser2481. Rictor expression in the gastric smooth muscle was increased in the DM6w and DM8w groups compared to the NC (both $P<0.01$), and DM4w groups (both $P<0.01$). also It was also higher in DM8w than in DM6w ($P<0.01$, Fig. 3C). The p-mTOR Ser^{2481}/mTOR ratio was increased in the DM8w group compared to the NC ($P<0.01$), DM4w ($P<0.05$), and DM6w ($P<0.05$) groups (Fig. 3D). The p-Akt Ser^{473}/Akt ratio was increased in both DM4w and DM8w compared to NC (both $P<0.01$). Also, the p-Akt Ser^{473}/Akt ratio was lower both in DM6w and DM8w than in DM4w (both $P<0.01$), and lower in DM8w than in DM6w ($P<0.01$, Fig. 3 E). The gastric smooth muscle cell surface expression of GLUT4 was increased in both DM4w and DM6w groups compared to NC ($P<0.01$, $P<0.05$). It was also lower in both DM6w and DM8w than in DM4 (both $P<0.01$) and lower in DM8w than in DM6w ($P<0.05$, Fig. 3F). These results suggested that the Rictor/mTORC2/Akt/GLUT4 pathway is involved in the regulation of energy metabolism in the gastric smooth muscle, alters energy supply to muscle, which in turn participates in the development of diabetic gastroparesis.

DISCUSSION

ATP is the most direct source of body energy, and 90% of it is produced by mitochondria (Barros & Baeza-Lehnert, 2019; Marklund *et al*., 2006; Korchazhkina *et al*., 1999). There are two main ways to generate ATP in the body: in the anaerobic state, glucose undergoes glycolysis, during which each molecule of glucose is converted to pyruvate and two molecules of ATP are produced, whereas in the aerobic state, pyruvate is completely metabolized to generate 32 molecules of ATP per molecule of glucose. In the present study, we found that in the diabetic state, starting from the sixth week following the induction of diabetes, there was an obvious reduction in gastric motility, which corresponds to the development of diabetic gastroparesis. The ATP concentration in the muscle did not change significantly with the progression of diabetes, but we observed an upward trend for ADP and AMP and ATP generation (ADP/ATP ratio),and a downward trend for energy charge. Energy charge represents the number of high-energy phosphate groups in the total adenylate system (the total concentration of ATP+ADP+AMP), which reflects the energy status of the cell. The above results imply deterioration in the energy status of the tissue. This may reflect a reduction in the aerobic metabolism of glucose because of partial replacement by other pathways, mitochondrial damage, or other factors. Although the substrate in the muscle is sufficient to ensure the conversion of ADP, it cannot reverse the trend of reduced synthesis. The specific explanation for this requires further study.

Diabetic gastroparesis has previously been shown to develop 6 weeks after the induction of diabetes in rats and is accompanied by changes in mTOR pathway (Zhang *et al*., 2017). mTOR is an atypical serine/threonine protein kinase that can integrate various extracellular signals, such as growth factors, nutrition, and energy status to regulate processes such as growth and the cell cycle. The mTOR complexes: mTORC1 and mTORC2 have different structures and functions, and different mTOR residues are phosphorylated in each complex (Saxton & Sabatini, 2017; Copp *et al*., 2009; Gnocchi *et al*., 2020). mTOR is activated by phosphorylation at multiple residues, including Ser2448 and Ser2481 (Saxton & Sabatini, 2017; Copp *et al*., 2009; Vazquez-Martin *et al*., 2012). Previous studies have shown that in mTORC1, mTOR is phosphorylated at Ser 2448, whereas in mTORC2, it is phosphorylated at Ser 2481 (Knudsen *et al*., 2020; Copp *et al*., 2009; Vazquez-Martin *et al*., 2012). mTORC1 contains rapamycin-sensitive Raptor, MLST8 a proline-rich Akt substrate of 40 kDa, (PRAS40), and DEP domain of mTOR-interacting protein (DEP domain-containing mTOR-interacting protein, DEPTOR); whereas, mTORC2 contains Rictor and MLST8, neither of which is sensitive to rapamycin, and SAPK interacting protein 1 (SIN1) (Saxton & Sabatini, 2017). Thus, Raptor and Rictor are characteristic proteins of the mTORC1 and mTORC2, respectively. In the present study we found that Rictor protein expression increased with the progression of diabetes, but mTOR expression was decreased at 8 weeks of diabetes. The phosphorylation of mTOR-Ser^{2481} requires intact mTORC2 (Knudsen *et al*., 2020), as the autophosphorylation of Ser^{2481} is proportional to the specific catalytic activity of mTORC2. By observing the changes in the p-mTOR Ser^{2481}/mTOR ratio, we found that the phosphorylation of mTOR at Ser^{2481} decreased prior to the development of gastroparesis, but increased after the onset of this complication, indicating that there are other factors that regulate the activation of mTORC2.

Rictor participates in glucose metabolism as an upstream effector of Akt, and when Rictor or mTORC2 is absent, glucose intolerance develops. While mTORC2 activates Akt, Akt can activate mTORC1 activity *via* Akt-(TSC1/2)-Ras homolog (RheB)-mTORC1, as was shown for brain tissue (Saxton & Sabatini, 2017). In another study, it was shown that the expression of Rictor in muscle cells is lower in a high-glucose environment and that the cells show lower insulin-induced glucose uptake, such that the glucose metabolism becomes disrupted (Mukaida *et al*., 2017). A reduction in Rictor expression also affects GLUT4 translocation, resulting in a reduction in glucose transport and deleterious effects on overall glucose and energy metabolism (Mukaida *et al*., 2017; Hasannejad *et al*., 2019; Khan & Kamal, 2019; Zhao *et al*., 2019). In this study, we found that the phosphorylation of Akt at Ser473 in the diabetic state gradually decreases, despite the increase in mTORC2 expression, which suggests that mTORC2 is not the dominant

regulator of Akt. GLUT4 translocation is mainly regulated by Akt, but Rictor is also required for this process. Although GLUT4 translocation is not mainly affected by mTORC2, there should be other ways to affect it.

GLUT4 translocation, and therefore glucose uptake by muscle cells, is promoted by Akt phosphorylation, but GLUT4 translocation decreases with the development of gastroparesis. In this study, the translocation of GLUT4 was found to be increased first and then decreased with disease progression. This result implies that in the diabetic state, glucose is taken up and used by cells also independently of insulin.

One of the limitations of this study is that we did not quantify the mRNA expression levels of the key proteins in the Rictor/mTORC2/Akt/GLUT4 pathway in the gastric smooth muscle of diabetic rats with RT-PCR.

CONCLUSIONS

Our findings documented that during the pathogenesis of diabetic gastroparesis, altered energy metabolism was present in the gastric smooth muscle of diabetic rats. is The change in energy metabolism was associated with the changes in protein expression and phosphorylation in the Rictor/mTORC2/Akt/Glut4 pathway, suggesting that this pathway may be involved in both the regulation of energy metabolism in the gastric smooth muscle of diabetic rats and in the development of diabetic gastroparesis.

Declarations

Conflicts of Interest. The authors declare no conflict of interest.

REFERENCES

Barros LF, Baeza-Lehnert F (2019) Perfect energy stability in neurons. Aging (Albany NY) **11**: 6622–6623. https://doi.org/10.18632/aging.102257

Copp J, Manning G, Hunter T (2009) TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser2481 is a marker for intact mTOR signaling complex 2. *Cancer Res* **69**: 1821–1827. https://doi.org/10.1158/0008-5472.CAN-08-3014

Cosimo E, Tarafdar A, Moles MW, Holroyd AK, Malik N, Catherwood MA, Hay J, Dunn KM, Macdonald AM, Guichard SM, O'Rourke D, Leach MT, Sansom OJ, Cosulich SC, McCaig AM, Michie AM (2019) AKT/mTORC2 inhibition activates FOXO1 function in CLL cells reducing B-Cell receptor-mediated survival. *Clin Cancer Res* **25**: 1574–1587. https://doi.org/10.1158/1078-0432.CCR-18-2036

Gnocchi D, Ellis ECS, Johansson H, Eriksson M, Bruscalupi G, Steffensen KR, Parini P (2020) Diiodothyronines regulate metabolic homeostasis in primary human hepatocytes by modulating mTORC1 and mTORC2 activity. *Mol Cell Endocrinol* **499**: 110604. https://doi.org/10.1016/j.mce.2019.110604

Hara K, Maruki Y, Long X, Yoshino K, Oshiro N, Hidayat S, Tokunaga C, Avruch J, Yonezawa K (2002) Raptor, a binding partner of target of rapamycin (TOR), mediates TOR action. *Cell* **110**: 177–189. https://doi.org/10.1016/s0092-8674(02)00833-4

Hasannejad M, Samsamshariat SZ, Esmaili A, Jahanian-Najafabadi A (2019) Klotho induces insulin resistance possibly through interference with GLUT4 translocation and activation of Akt, GSK3β, and PFKfβ3 in 3T3-L1 adipocyte cells. *Res Pharm Sci* **14**: 369–377. https://doi.org/10.4103/1735-5362.263627

Khan S, Kamal MA (2019) Wogonin alleviates hyperglycemia through increased glucose entry into cells *via* AKT/GLUT4 pathway. *Curr Pharm Des* **25**: 2602–2606. https://doi.org/10.2174/1381612825666190722115410

Kleinert M, Sylow L, Fazakerley DJ, Krycer JR, Thomas KC, Oxbøll AJ, Jordy AB, Jensen TE, Yang G, Schjerling P, Kiens B, James DE, Ruegg MA, Richter EA (2014) Acute mTOR inhibition induces insulin resistance and alters substrate utilization *in vivo*. *Mol Metab* **3**: 630–641. https://doi.org/10.1016/j.molmet.2014.06.004

Kimoloi S (2018) Modulation of the *de novo* purine nucleotide pathway as a therapeutic strategy in mitochondrial myopathy. *Pharmacol Res* **138**: 37–42. https://doi.org/10.1016/j.phrs.2018.09.027

Knudsen JR, Fritzen AM, James DE, Jensen TE, Kleinert M, Richter EA (2020) Growth factor-dependent and -independent activation of mTORC2. *Trends Endocrinol Metab* **31**: 13–24. https://doi.org/10.1016/j.tem.2019.09.005

Korchazhkina O, Wright G, Exley C (1999) No effect of aluminium upon the hydrolysis of ATP in the coronary circulation of the isolated working rat heart. *J Inorg Biochem* **76**: 121–126. https://doi.org/10.1016/s0162-0134(99)00123-3

Marklund N, Salci K, Ronquist G, Hillered L (2006) Energy metabolic changes in the early post-injury period following traumatic brain injury in rats. *Neurochem Res* **31**: 1085–1093. https://doi.org/10.1007/s11064-006-9120-0

Mukaida S, Evans BA, Bengtsson T, Hutchinson DS, Sato M (2017) Adrenoceptors promote glucose uptake into adipocytes and muscle by an insulin-independent signaling pathway involving mechanistic target of rapamycin complex 2. *Pharmacol Res* **116**: 87–92. https://doi.org/10.1016/j.phrs.2016.12.022

Saxton RA, Sabatini DM (2017) mTOR Signaling in growth, metabolism, and disease. *Cell* **168**: 960–976. https://doi.org/10.1016/j.cell.2017.02.004

Vazquez-Martin A, Sauri-Nadal T, Menendez OJ, Oliveras-Ferraros C, Cufí S, Corominas-Faja B, López-Bonet E, Menendez JA (2012) Ser2481-autophosphorylated mTOR colocalizes with chromosomal passenger proteins during mammalian cell cytokinesis. *Cell Cycle* **11**: 4211–4221. https://doi.org/10.4161/cc.22551

Manuel Wilbring, Annette Ebner, Katrin Schoenemann, Michael Knaut, Sems Malte Tugtekin, Birgit Zatschler, Thomas Waldow, Konstantin Alexiou, Klaus Matschke, Andreas Deussen (2013) Heparinized blood better preserves cellular energy charge and vascular functions of intraoperatively stored saphenous vein grafts in comparison to isotonic sodium-chloride-solution. *Clin Hemorheol Microcirc* **55**: 445–455. https://doi.org/10.3233/CH-131781

Zhang MH, Jiang JZ, Cai YL, Piao LH, Jin Z (2017) Significance of dynamic changes in gastric smooth muscle cell apoptosis, PI3K-AKT-mTOR and AMPK-mTOR signaling in a rat model of diabetic gastroparesis. *Mol Med Rep* **16**: 1530–1536. https://doi.org/10.3892/mmr.2017.6764

Zhao P, Tian D, Song G, Ming Q, Liu J, Shen J, Liu QH, Yang X (2019) Neferine promotes GLUT4 expression and fusion with the plasma membrane to induce glucose uptake in L6 cells. *Front Pharmacol* **10**: 999. https://doi.org/10.3389/fphar.2019.00999