The difference between steroid diabetes mellitus and type 2 diabetes mellitus: A whole-body 18F-FDG PET/CT study

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Qingqing Zhao
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital
ORCiD: https://orcid.org/0000-0002-7721-8273

Jinxin Zhou
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Yu Pan
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Huijun Ju
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Liying Zhu
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Yang Liu
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Yifan Zhang
Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

zhang_yifan@126.com Corresponding Author
ORCiD: https://orcid.org/0000-0001-6488-6232

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Abstract

Background
Steroid diabetes mellitus (SDM) is a metabolic syndrome caused by an increase in glucocorticoids, and its pathogenesis is unclear. $^{18}$F-FDG PET/CT can reflect the glucose metabolism of tissues and organs under living conditions, and plays an important role in diabetes research. Here, PET/CT imaging of SDM and type 2 diabetes mellitus (T2DM) rats was used to observe the changes of glucose metabolism in major glucose metabolism organs and immunohistochemical analysis to explore the possible pathogenesis of SDM.

Results
The SDM rat model was successfully established, which showed increased FBG and insulin levels; $^{18}$F-FDG PET/CT imaging showed increased FDG uptake in skeletal muscle, but no significant increase in liver uptake (15d); Immunohistochemistry showed that islet $\alpha$-cell and $\beta$-cell proliferation, GLUT-4 and IRS-1, PI3Kp85$\alpha$ expression in skeletal muscle increased, and glycogen storage in liver and skeletal muscle increased. T2DM rats showed atrophy of pancreatic islet $\beta$ cells and decreased insulin levels; Significantly reduced FDG uptake and glycogen storage in skeletal muscle and liver; IRS-1 expression in skeletal muscle decreased, and GLUT-4 and PI3Kp85$\alpha$ did not change significantly.

Conclusion
The pathogenesis of SDM is different from that of T2DM. The increased glucose metabolism of skeletal muscle may be related to the increased compensatory secretion of insulin; glucocorticoids promote the proliferation of islet $\alpha$ cells and cause the increase of gluconeogenesis in the liver may be the cause of its increased blood glucose.

Background
Glucocorticoids are of key clinical use, due to their effective anti-inflammatory, anti-allergic, and immunosuppressive effects(1). However, excessive glucocorticoids (endocrine corticosteroid secretion or exogenous glucocorticoid intake) in the body often leads to glucose metabolism disorders, a condition termed steroid diabetes mellitus (SDM). Studies have shown that 32.2% of patients without a clear history of diabetes have increased blood glucose after using glucocorticoids (random blood glucose > 11.1 mmol/L) (2). The incidence of impaired glucose tolerance in Cushing's syndrome (CS)
patients with excessive endocrine glucocorticoids can be as high as 70% (3). The occurrence of SDM seriously affects the survival rate and quality of life of patients (4–6).

At present, domestic and foreign studies generally classify SDM as type 2 diabetes mellitus (T2DM). It is thought that the occurrence of SDM is related to the damage of islet β cell function, and insulin resistance in major glucose metabolism organs, such as skeletal muscle, liver, and fat caused by glucocorticoids (7–9). However, clinical diagnosis and treatment has revealed that SDM patients differ from T2DM patients, and some SDM patients can return to normal blood glucose levels after exposure to excess glucocorticoids. Clinical studies by Giordano et al. demonstrated that the insulin sensitivity index-Matsuda (ISI-Matsuda) and homeostasis model assessment of insulin resistance (HOMA-IR) were not significantly different between CS diabetic and CS non-diabetic patients (10). The mechanism of glucocorticoid-induced diabetes requires further investigation.

Previous studies have shown that both acute and chronic glucocorticoids exposure can inhibit insulin release in a dose-dependent manner in rodents (11, 12). In clinical studies, Van et al. found that after taking prednisolone, impaired glucose tolerance, reduced C peptide secretion stimulated by arginine, and islet cell dysfunction in healthy volunteers (13). However, some studies reported that islet β cell function and mass increased following glucocorticoid treatment, due to the body's compensatory effect (14, 15). Skeletal muscle is the main organ of insulin-mediated glucose uptake (> 80%) (16). Studies show that glucocorticoids can directly interfere with insulin signaling in skeletal muscle cells, inhibit glucose transporter 4 (GLUT4) expression, and reduce insulin-stimulated glucose uptake and glycogen synthesis in isolated skeletal muscle (17, 18). However, some studies have shown that after dexamethasone treatment in rats, the expression of GLUT4 in skeletal muscle does not decrease, and the glycogen content in skeletal muscle and liver increases (19). Studies have shown that glucocorticoids also seem to cause insulin resistance by promoting liver gluconeogenesis and adipogenesis (20).

Taken together, current research on the pathogenesis of SDM is limited to in vitro conditions or the assessment of overall glucose metabolism, and neither can reflect the glucose metabolism of tissues and organs under living conditions. Due to 18F-FDG having similar biochemical properties to glucose,
it can enter cells through the glucose transporter on the cell membrane, where it is phosphorylated to 6-PO$_4$-$^{18}\text{F-FDG}$ by hexokinase, and it remains in the cell. $^{18}\text{F-FDG}$ PET/CT imaging can reflect glucose metabolism of tissues and organs in the body\textsuperscript{(21, 22)}. At present, $^{18}\text{F-FDG}$ PET imaging has been used to study the glucose metabolism of organs such as the liver, fat, skeletal muscle, and myocardium\textsuperscript{(23–25)}, and the results show that this approach allows for the effective evaluation of glucose uptake by these organs.

The aim of this study was to investigate the pathogenesis of SDM and how it differs from T2DM, through the establishment of SDM\textsuperscript{(26, 27)} and T2DM rat models and subsequent $^{18}\text{F-FDG}$ micro-PET/CT imaging and immunohistochemical analyses.

Materials And Methods

**Animals care**

The experimental rats were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China), and housed in the laboratory animal centre of Ruijin hospital, which is affiliated to the medical college of Shanghai Jiao Tong University. The temperature was maintained between 22–24°C, and rats were subjected to a 12/12 h light/ dark cycle (lights on at 07:00 am) and provided food (Shanghai SLAC company, China) and water ad libitum. The animal study was approved by the Ethics Committee of Shanghai Jiao Tong University School of Medicine (Shanghai, China) and was conducted in accordance with the ethical principles governing animal welfare, rearing, and experimentation.

**Dexamethasone treatment and experimental method**

The basal body weight and fasting blood glucose (FBG) values of 35 male Wistar rats (250–300 g) were measured after one week of adaptive feeding. The experimental rats were randomly divided into 2 groups, 20 in the dexamethasone treatment group (SDM), and 15 in the control group (CTL). SDM rats were injected intraperitoneally with dexamethasone (Sigma–Aldrich, USA) (10 mg/kg) daily\textsuperscript{(27)}, and 5 were used for orbital blood collection, 5 for glucose tolerance tests, and 10 for PET/CT imaging. CTL rats were intraperitoneally injected with a corresponding dose of normal saline daily, and 5 were used for orbital blood collection, 5 for glucose tolerance tests, and 5 for PET/CT imaging. In the SDM
and CTL groups, changes in body weight and FBG were monitored every other day. Orbital blood collection, glucose tolerance tests, and PET imaging were performed on days 0, 3, 7, 11, and 15 of the experimental time frame.

The basal body weight and FBG values of 10 male GK rats, spontaneous T2DM rats (100–150 g), were measured after one week of adaptive feeding with ordinary feed, and the first imaging was performed. The administration of the high-fat diet increased the disease process, and changes in body weight and blood glucose were monitored once a week, and small animal imaging was performed every 1-2 months.

**FBG, serum insulin, and intraperitoneal glucose tolerance test**

After 12 h fasting, blood was drawn from the tail tip of the rats, and their FBG was measured using a Bayan automatic blood glucose meter(28) (Contour TM TS, Bayer, Germany). Similarly, blood was collected from the orbits of the rats, under anesthesia(29). The blood was centrifuged at 2000 × g at 4°C for 20 min, before the supernatant was collected and stored at -80°C. Serum insulin was detected with a rat insulin ELISA kits (30) (Rat Insulin ELISA Kit, Crystal Chem, USA).

Intraperitoneal glucose tolerance test: after overnight fasting, the rats were given a glucose load (1 g/kg) by intraperitoneal injection of a 50% glucose solution. The blood glucose was measured at 0, 30, 60, 90, and 120 min following glucose loading. For statistical analyses, the blood glucose change curves were plotted and the area under the curve (AUC) calculated(31).

**Micro PET/CT imaging**

After 12 h overnight fasting, rats were anaesthetized with 3% pentobarbital sodium (Sigma-Aldrich, USA) (30 mg/kg) by intraperitoneal injection. The tail vein was injected with $^{18}$F-FDG at a dose of approximately 7.4 MBq. After 30 min, rats were fixed in the prone position on the center of a Micro PET/CT (Inveon MM Platform, Siemens Preclinical Solutions, Knoxville, Tennessee, USA) scan bed field of view (FOV), and scanned under anesthesia. The PET/CT equipment has a resolution of 1.5 mm, an aperture of 5.7 cm, and an axial FOV of 8.5 cm. The micro PET/CT equipment acquisition workstation was Inveon Acquisition Workplace (IAW) 1.5.0.28. A new workflow was established before data acquisition, including CT Acquisition, Reconstruction, PET Acquisition, PET Histogram, and PET
Reconstruction. The static scan data was collected under the conditions of 80 kV voltage, 500 μA current, and 1100 ms exposure for 10 min, and then PET data was collected. The collected data was reconstructed with IAW software through attenuation correction, and the three-dimensional ordered subsets maximization algorithm (OSEM3D) was used to reconstruct the coronal, transverse, and sagittal tomographic images for analysis. The reconstructed images were obtained using Siemens Inveon Research Workplace (IRW) 3.0 to obtain 3D Regions of Interest (ROI). In this study, the skeletal muscle comprised the upper limb epitrochlearis muscle(32), and the upper right portion of the liver was utilized. Finally, the %ID/g max value of the ROI was obtained for quantitative analysis(33).

**Quantitative RT-PCR**

RNA extraction and cDNA synthesis from muscle were performed using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and PrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, China Da Lian), respectively, according to the manufacturers’ protocols. Total RNA (500 ng) was amplified on a StepOne Fast Real-Time PCR System (Thermo Fisher Scientific), using TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, China Da Lian) for real-time PCR after cDNA synthesis. The standard curve for quantification was derived as per a modified version of a previously described method. Fold change of the gene expression was calculated by 2−ΔΔCt relative to the internal reference gene (glyceraldehyde3-phosphate dehydrogenase, GAPDH). The primer sequences used are as follows:

- GLUT4: forward 5′-GGGCTGTGAGTGAGTGCTTTC-3′, reverse 5′- CAGCGAGGCAAGGCTAGA-3′; insulin receptor substrate 1 (IRS-1): forward 5′-ATGTGGAAATGGCTCGGA-3′, reverse 5′-TAAGGCAGCAAAGGGTAGGC-3′; Phosphatidylinositol 3-kinase (PI3K)-p85α: forward 5′- TTAAACGCGAAGGGAACGA-3′, reverse 5′-CAGTCTCCTCCTGCTGATC-3′; GAPDH: forward 5′-AGTCGGTGTGAACGGATTTG-3′, reverse 5′-TGTAGACCATTGAGTTGAGGTCA-3′.

**Histological analyses**

After the rats were euthanized, the excised pancreatic tissue and epitrochlearis muscle of the upper limbs were immersed in 4% paraformaldehyde (Sigma-Aldrich, USA) for 48 h. Tissues were washed in 70% ethanol, embedded in paraffin, and sectioned onto glass slides. The slides were dewaxed with xylene, dehydrated with ethanol, and washed with PBS. The slides were then incubated in a hydrogen
peroxide blocking solution for 10 min at 18-25°C to block endogenous peroxidase activity. Anti-
masking/epitope retrieval of the antigen was performed by high pressure heating of 1 mMol Tris-EDTA (pH 9.0). Slides were incubated in a protein blocking solution (Sigma-Aldrich, USA), before the pancreatic tissue was tested for insulin (1:100, ab7842, Abcam, CA, USA) and glucagon (1:200, ab10988, Abcam, CA, USA). Skeletal muscle was tested for GLUT4 (1:500, ab654, Abcam, CA, USA), IRS-1 (1:100, ab52167, Abcam, CA, USA), and PI3Kp85α (1:100, ab182651, Abcam, CA, USA). After overnight incubation at 4°C, the slides were immunostained with secondary antibodies from EnVision reagent (HRP/rabbit and mouse, Dako, K5007 kit, Denmark). DAB was added and allowed to develop under microscope observation until brown staining was visible. Slides were counterstained with hematoxylin (Sigma-Aldrich, USA). Representative slides of insulin and glucagon were used to quantify the mass and islet area of islet β and α cells.

**Statistical analyses**

Results are presented as mean ± standard error of the mean (unless otherwise stated). Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). *, statistical significance where P<0.05; **, statistical significance where P<0.01; ***, statistical significance where P<0.001; ****, statistical significance where P<0.0001.

**Results**

**Differences in body weight, FBG, glucose tolerance, and fasting insulin between SDM and T2DM rats**

After SDM rats were injected intraperitoneally with 10 mg/kg of dexamethasone daily, their weight gradually decreased over time (Fig. 1a). FBG dropped after a transient rise on the 3rd day of administration, and slowly increased to 9.8 mmol/L from day 7 to 15, which was significantly higher than that of the CTL rats (Fig. 1b). Glucose tolerance test results show the AUC of SDM rats is significantly higher than that of the CTL rats (Fig. 1c). SDM rats showed significant hyperinsulinemia after dexamethasone injection. Specifically, fasting insulin increased significantly on the 3rd day of dexamethasone treatment, gradually decreased from day 3 to 11, and increased again on the 15th day; all timepoints were significantly higher than the CTL rats (Fig. 1d).
As GK rats develop diabetes slowly, high-fat diets were used to accelerate their disease progression. The modelling time of T2DM model lasted 4 months, and the FBG values were similar to that of the SDM rats (day 15). The weight of the T2DM rats gradually increased over disease progression (Fig.2a). The FBG gradually increased with time, before stabilizing (about 9.4 mmol/L) (Fig.2b). Fasting insulin showed a decreasing trend, and there was a significant difference between 0 and 4 months (Fig.2c). SDM rats (day 15 of dexamethasone treatment) matched T2DM rats (high-fat diet 4 months) FBG levels. T2DM rats showed significant weight gain and fasting insulin levels decreased, while SDM rats showed weight loss and fasting insulin levels that increased significantly.

**Difference in glucose uptake in the skeletal muscle and liver of SDM and T2DM rats**

$^{18}$F-FDG micro PET/CT imaging results showed that the glucose uptake by skeletal muscle in SDM rats gradually increased over time, reaching a peak on day 11, with a slight decrease on day 15; the %ID/g of glucose uptake in skeletal muscle on days 7, 11, and 15 is significantly higher than at day 0 (Fig.3a). The glucose uptake by skeletal muscle in T2DM rats gradually decreased over time, and there was a statistical difference between 1, 3, and 4 months compared to 0 months (Fig.3b). Hepatic glucose uptake was also different between SDM and T2DM rats. SDM liver glucose uptake did not change significantly over time, and gradually increases. The increase in liver glucose uptake on day 7 was significantly different to day 0 (Fig.3c). T2DM rat liver glucose uptake gradually decreased, and it was significant at 1, 3, and 4 months compared to 0 months (Fig.3d). The overall level of liver glucose uptake in SDM rats during disease progression was lower than that in T2DM rats.

**Differences in SDM and T2DM rat skeletal muscle expression of GLUT4 and insulin signaling pathways, and glycogen contents between skeletal muscle and the liver**

Glycogen (PAS) staining analysis in the liver and skeletal muscle of SDM rats (15 days) and T2DM rats (4 months) showed that SDM rats had higher glycogen levels in the liver and skeletal muscle than CTL and T2DM rats (Fig.4a). Immunohistochemical analysis of GLUT4, PI3Kp85α, and IRS-1 in the skeletal muscle of SDM rats (15 days) and T2DM rats (4 months) showed that GLUT4, IRS-1, and PI3Kp85α were higher in the skeletal muscle of SDM rats than that of CTL and T2DM rats; there was no significant difference in the expression of GLUT4, IRS-1, and PI3Kp85α in skeletal muscle between
T2DM and CTL rats (Fig. 4b). RNA results showed that the levels of GLUT4, IRS-1, and PI3Kp85α mRNA in the skeletal muscle of SDM rats were significantly higher than those of CTL rats (P < 0.05, 0.001, and 0.01, respectively) (Fig. 4c). The levels of GLUT4 and PI3Kp85α mRNA in the skeletal muscle of T2DM rats were not significantly different to CTL rats, and expression of IRS-1 was significantly lower than CTL rats (P < 0.01) (Fig. 4c).

Morphological pancreatic differences between SDM and T2DM rats

Immunohistochemical analysis of pancreatic insulin and glucagon in SDM rats (15 days) and T2DM rats (4 months) (Fig. 5) showed that the total area of pancreatic islet β cells in SDM rats increased, but it was not significantly different to the CTL rats (P=0.19). The total area of pancreatic β cells in T2DM rats was significantly lower than that in the CTL group (P<0.05), and there was no significant difference compared to SDM rats (P=0.06). The total area of islet α cells in SDM rats increased; while there was no significant difference compared to CTL rats (P=0.10), there was compared to T2DM rats (P<0.05). The total area of islet α cells in T2DM rats was not significantly different to CTL rats (P=0.72).

Discussion

This is the first study to report on the differences in glucose uptake in skeletal muscle and the liver during the progression of SDM and T2DM using a molecular imaging approach. The study found that with the same level of hyperglycemia, the fasting insulin levels in SDM rats was significantly increased, as was pancreatic β cell proliferation. T2DM rats were observed to have lower fasting insulin levels, and the total area of islet β cells decreased and showed impaired function. PET/CT results showed that glucose uptake in the skeletal muscle of SDM rats increased, which was accompanied by an up-regulation of PI3Kp85α, IRS-1, and GLUT4; the glycogen content of the liver and skeletal muscle also increased in SDM rats. T2DM rats' skeletal muscle and liver glucose decreased, along with a down-regulation of IRS-1 in skeletal muscle, unchanged GLUT4 and PI3Kp85α expression, and decreased glycogen content in the liver and skeletal muscle. Pancreatic β cells play a vital role in glucose metabolism, and β cell dysfunction is been considered to be a key factor in the pathogenesis of T2DM. In vitro studies show that glucocorticoids can reduce β
cell glucose uptake and phosphorylation, thereby reducing ATP synthesis and Ca\(^{+}\) influx, leading to reduced insulin biosynthesis and release(11, 34). In addition, glucocorticoids can reduce \(\beta\) cell mass by inducing apoptosis(35, 36). To the opposite, studies by Rafacho et al. showed that with high-dose dexamethasone treatment, rat pancreatic islet \(\beta\) cells underwent adaptive changes, the ability of the islets to respond to glucose increased, and insulin compensated secretion increased(37, 38). Protzek et al. studies have shown that rats and mice treated with dexamethasone in vivo have enhanced \(\beta\) cell function (increased glucose-stimulated insulin secretion) and increased mass (\(\beta\) cell compensatory hyperplasia) (39). In this study, unlike T2DM, SDM manifested as islet \(\beta\) cell compensatory hyperplasia and increased insulin secretion, indicating that changes in islet \(\beta\) cell mass are not the main cause of SDM. However, it is interesting to note that pancreatic \(\alpha\) cell hyperplasia was also observed in this study. Previous studies have shown that glucocorticoids can cause pancreatic \(\alpha\)-cell proliferation and induce hyperglucagonemia, leading to increased blood glucose and \(\beta\) cell generation compensatory hyperplasia. Glucocorticoid-induced \(\alpha\) cell proliferation may be one of the causes of SDM(40, 41). In addition, glucocorticoids may affect the release of other hormones in the pancreas, such as somatostatin, amylin, and ghrelin, which may also be a cause of glucocorticoid-induced diabetes(42).

Skeletal muscle is the main organ for insulin-dependent glucose uptake. It is responsible for 80% of glucose intake after meals and is the body's largest glycogen storage organ(11). In skeletal muscle, after insulin binds to its receptor, it activates IRS-1, which in turn activates downstream PI3Kp85\(\alpha\). Protein kinase B (PKB) is subsequently activated by phosphorylation. Once activated, GLUT4 translocation from intracellular vesicles to the cell membrane is enhanced, glucose uptake increased, and PKB phosphorylates glycogen synthase kinase-3 (GSK), deactivating the kinase. GSK3 inactivation promotes glycogen synthase activation and increases glycogen synthesis(19). Studies show that glucocorticoids can induce insulin resistance by directly interfering with insulin signaling in skeletal muscle(43). After treatment with dexamethasone in rats, Ruzzin et al. found that dexamethasone may reduce insulin-stimulated PKB and GSK-3 phosphorylation in skeletal muscle(19). Burén et al. also found that dexamethasone treatment reduced the expression of IRS-1 and PKB in the skeletal
muscle insulin signaling pathway (44). Both studies found that insulin-stimulated glucose uptake and glycogen synthesis in skeletal muscle were reduced, but these results were limited to in vitro conditions, and the expression of insulin-related proteins in skeletal muscle was also inconsistent. Ruzzin et al. have observed increased expression of PI3Kp85α, PKB, and GSK-3 in skeletal muscle (19). Moreover, in both studies, a phenomenon consistent with this study was observed, with increased in GLUT4 expression and the glycogen content in skeletal muscle, suggesting that although glucocorticoids can directly act on the insulin signaling pathway and cause skeletal muscle insulin resistance; however, due to increased insulin secretion, glucose uptake and glycogen content increased in skeletal muscle (45). In T2DM rats, lower insulin levels and insulin resistance may be an important cause of reduced glucose uptake in skeletal muscle (46, 47). Here, it was found that under the same blood glucose levels, compared to T2DM rat skeletal muscle, SDM rat skeletal muscle had a stronger capacity for glucose uptake and utilization. The change in glucose uptake of skeletal muscle is not the main reason for the increase in blood glucose in SDM.

The liver is the main organ of gluconeogenesis and plays an important role in glucose metabolism and blood glucose regulation. When the body has sufficient energy, excess blood glucose absorbed from the digestive tract is stored in the liver in the form of glycogen. In the state of starvation or fasting, gluconeogenesis in the liver increases, and glycogen is broken down and phosphorylated into glucose 6-phosphate, which is then converted into glucose and released into the blood under the action of glucose-6-phosphatase for the body's energy metabolism. Blood glucose is an important factor affecting liver glucose uptake. When the blood glucose rises, it will not only competitively inhibit the uptake of 18F-FDG by liver cells, but also feedback inhibit the activity of glucose-6-phosphatase in liver cells; 6-phosphate-FDG cannot rapidly release the phosphate to release the glucose into the blood, and thus it accumulates in the liver, and there is an observed increase in liver 18F-FDG uptake (48). In the present study, the initial increase in T2DM rat blood glucose coincided with a significant increase in liver 18F-FDG uptake. However, during the development of T2DM disease, with the increase in body weight, the decrease of insulin levels, the development of fatty liver, and the
occurrence of insulin resistance, the liver $^{18}$F-FDG gradually decreased (49). In SDM rats, although their blood glucose was also high, as glucocorticoids can promote liver gluconeogenesis, the rate-limiting enzyme phosphoenolpyruvate carboxy kinase and glucose-6-phosphatase activity increased, leading to increased liver glucose output (20), and a liver $^{18}$F-FDG uptake lower than in T2DM rats. The uptake of glucose in the liver by glucose transporter 2 (GLUT2) is independent of insulin, and the effect of insulin on $^{18}$F-FDG uptake in the liver of SDM rats is not significant (50, 51). Taken together, unlike T2DM, the increased glucose output of the liver plays a more important role in the increase of blood glucose in SDM.

As a molecular imaging detection instrument, PET/CT can reflect the metabolic status of tissues and organs under living conditions. In this study, one limitation was that a glucose clamp test was not performed during PET/CT imaging. However, our study conducted a parallel control study with T2DM rats, and found that SDM rats have comparatively increased skeletal muscle glucose uptake and lower liver glucose uptake, suggesting that the main reason for the increase in blood glucose in SDM may be the increase in liver gluconeogenesis and increased glucose output.

**Conclusion**

PET/CT imaging showed increased skeletal muscle glucose uptake and lower liver glucose uptake in SDM rats. The expression of the insulin signaling factors IRS-1 and PI3Kp85α increased, glucose uptake mediated by GLUT4 in SDM skeletal muscle increased, liver and skeletal muscle glycogen content increased, islet α cell number increased, and islet β cell compensatory hyperplasia was evident. Therefore, the pathogenesis of SDM is different to that of T2DM. The pathogenesis of SDM may be related to increased gluconeogenesis in the liver caused by the glucagon-like effect of glucocorticoids. These findings provide new insight into the pathogenesis of SDM, which is useful in the development of novel treatment strategies.

**Abbreviations**
Declarations

Ethics approval and Consent to participate
The animal study was approved by the Ethics Committee of Shanghai Jiao Tong University School of Medicine (Shanghai, China) and was conducted in accordance with the ethical principles governing animal welfare, rearing, and experimentation.

Consent for publication
Not applicable

Availability of data and material
All data generated or analysed during this study are included in this published article

Competing interests
The authors have no conflicts of interest to declare.

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Authors' contributions
Jinxin Zhou and Yifan Zhang proposed initial experimental ideas. Qingqing Zhao completed the experimental design with the help of Yifan Zhang, Yu Pan, and Jinxin Zhou. Qingqing Zhao completed the entire experiment with the help of Yu Pan, Jinxin Zhou, Huijun Ju, Liying Zhu, and Yang Liu. Qingqing Zhao completed the article writing with the help of Yifan Zhang, Yu Pan and Jinxin Zhou.

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Figures

**Figure 1**

Metabolic changes in SDM rats (weight, FBG, glucose tolerance, and fasting insulin). a: Rat weight over time (SDM, n = 10; CTL, n = 7); b: FBG over time (SDM, n = 10; CTL, n = 7); c: Area under the curve for the glucose tolerance test over time (n = 4); d: Fasting insulin over time (n = 4). * Compared to CTL rats.
Figure 2

Metabolic changes in T2DM rats (weight, FBG, and fasting insulin). a: T2DM rat weight over time (n = 6); b: T2DM rat FBG over time (n = 4); c: T2DM rat fasting insulin over time (n = 4). * Compared to month 0.
Figure 3

Glucose uptake (% ID/g max) of skeletal muscle and liver in PET/CT imaging over time. a: Glucose uptake by skeletal muscle in SDM rats; b: Glucose uptake by skeletal muscle in T2DM rats; c: Glucose uptake by the liver in SDM rats; d: Glucose uptake by the liver in T2DM rats. n = 4, * Indicates that the statistical analysis of SDM rats is compared to day 0, and that of T2DM rats is compared to month 0.
Immunohistochemistry and RNA analysis of skeletal muscle and liver in CTL, SDM, and T2DM rats. a: Analysis of glycogen content (PAS) in the liver and skeletal muscle of CTL, SDM, and T2DM rats; b: Immunohistochemical analysis of GLUT4, PI3Kp85α, and IRS-1 in the skeletal muscle of CTL, SDM, and T2DM rats; c: Analysis of RNA expression levels of GLUT4, PI3Kp85α, and IRS-1 genes in the skeletal muscle of CTL, SDM, and T2DM rats. n = 4, * compared to CTL rats.
Immunohistochemical results of pancreatic tissue. Immunohistochemical results of insulin and glucagon in pancreatic tissue of CTL, SDM, and T2DM rats (n = 4). * Compared to CTL rats; # compared to SDM rats.