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The mitochondrial-derived peptide MOTS-c relieves hyperglycemia and insulin resistance in gestational diabetes mellitus

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ABSTRACT

The most common complication during pregnancy, gestational diabetes mellitus (GDM), can cause adverse pregnancy outcomes and result in the mother and infant having a higher risk of developing type 2 diabetes after pregnancy. However, existing therapies for GDM remain scant, with the most common being lifestyle intervention and appropriate insulin treatment. MOTS-c, a mitochondrial-derived peptide, can target skeletal muscle and enhance glucose metabolism. Here, we demonstrate that MOTS-c can be an effective treatment for GDM. A GDM mouse model was established by short term high-fat diet combined with low-dose streptozotocin (STZ) treatment while MOTS-c was administrated daily during pregnancy. GDM symptoms such as blood glucose and insulin levels, glucose and insulin tolerance, as well as reproductive outcomes were investigated. MOTS-c significantly alleviated hyperglycemia, improved insulin sensitivity and glucose tolerance, and reduced birth weight and the death of offspring induced by GDM. Similar to a previous study, MOTS-c also could activate insulin sensitivity in the skeletal muscle of GDM mice and elevate glucose uptake in vitro. In addition, we found that MOTS-c protects pancreatic β-cell from STZ-mediated injury. Taken together, our findings demonstrate that MOTS-c could be a promising strategy for the treatment of GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance with onset or first recognition during pregnancy [1,2]. Currently, GDM affects 10.5–24.2% of pregnancies worldwide and 11.9% of pregnancies in China [3,4]. GDM begets short- and long-term serious adverse implications for the health of mother, developing fetus, and even other offspring [2]. Specifically, GDM can cause fetal macrosomia, obstructed shoulder delivery, postpartum hemorrhaging, neonatal asphyxia or death in the short term [2]. Even worse, women with GDM and their offspring have an increased risk to develop type 2 diabetes mellitus, obesity or other metabolic diseases later in life [5]. About 70.0% of women with GDM will develop diabetes within 22–28 years after pregnancy [6]. Hence, GDM contributes to a vicious intergenerational cycle from mother to child and impacts the health of the entire global population.

GDM is characterized by insulin resistance and insulin insufficiency during pregnancy [1,2]. As pregnancy progresses, the placenta gradually increases the secretion of anti-insulin hormones (prolactin, progesterone, estradiol and placental growth hormone), which results in an increased insulin demand from pancreatic β cells [2]. In normal pregnancies, there is a physiological increase in β-cell proliferation and insulin secretion to adapt to aggravated insulin resistance [7]. However, abnormally increased levels of anti-insulin hormones and/or β-cell dysfunction in pregnant women results in a relative deficiency of insulin, ultimately leading to an imbalance of glucose metabolism and GDM [8]. The current first line of treatment for GDM is based on nutritional therapy and exercise intervention. For those whom lifestyle interventions do not facilitate effective glycemic control, additional insulin therapy is often prescribed [9]. However, there are still drawbacks, such as poor treatment outcomes, lack of universal compliance, and/or an increasing levels of anxiety [10]. Therefore, a kind of new treatment

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from the perspective of improving insulin sensitivity or protecting pancreas islet function is extremely important for the short- and long-term prognosis of GDM.

Mitochondrial open reading frame of the 12S rRNA type-c (MOTS-c) is a recently identified mitochondrial-derived peptide consisting of 16 amino acids, which is highly conserved between humans and rodents [11]. According to previous reports, MOTS-c can be secreted into the circulation and act in a cell-autonomous and hormonal manner [12]. MOTS-c targets skeletal muscle to promote insulin sensitivity and prevent diet-induced obesity and insulin resistance in C57BL/6J mice [11]. Notably, the level of circulating MOTS-c was significantly decreased in young obese males [13], negatively correlated with insulin resistance in lean individuals [14], and it was also shown to be decreased in type 1/2 diabetes (T1/2D) patients [15]. In addition, MOTS-c can be induced by exercise and enhances physical performance in mice of different age via regulating muscle homeostasis [16]. Recently, Joseph C et al. reported that MOTS-c can prevent pancreatic islet destruction and suppresses autoimmune diabetes [17]. All of these reports indicate that MOTS-c acts as an endocrine factor to regulate the metabolism within or between cells. However, there have been no relevant studies regarding the functional aspects of MOTS-c in GDM so far, and it is still unknown whether MOTS-c is involved in insulin resistance and blood glucose regulation in pregnancy.

In present study, we observed that GDM women had lower levels of circulating MOTS-c than healthy controls, suggesting that MOTS-c may contribute to the development of GDM. To test this hypothesis, we evaluated the function of MOTS-c using a GDM mouse model and found that MOTS-c could effectively alleviate hyperglycemia and improve insulin tolerance and glucose metabolism, as well as improving these outcomes in offspring after MOTS-c treatment. In parallel with a previous study, we also explored whether the adenosine monophosphate-activated protein kinase (AMPK) signal was activated in skeletal muscle by MOTS-c. Furthermore, we revealed that MOTS-c promoted β-cell proliferation in GDM mice to resist streptozotocin (STZ)-induced dysfunction.

2. Materials and methods

2.1. Human studies

Twenty normal glucose-tolerant pregnant women and 20 pregnant women with GDM were recruited. A diagnosis of GDM was based on the 75-g oral glucose tolerance test (OGTT), together with the IADPSG (International Association of Diabetes and Pregnancy Study Group) criteria. Specifically, pregnant women with fasting glucose levels $\geq$ 5.10 mmol/L, 1-h glucose levels $\geq$ 10.00 mmol/L or 2-h glucose levels $\geq$ 8.50 mmol/L in a OGTT were diagnosed as having GDM. Inclusion criteria were patients needed to be 22–35 years of age, have complete medical records for both the pregnant women and from a single pregnancy. Exclusion criteria included pregnancy complicated with diabetes or chronic hypertension, taking drugs affecting blood glucose metabolism during pregnancy, or a pregnancy complicated with thyroid disease or autoimmune disease. Blood samples were collected when screening for GDM using OGTT at 20–24 weeks of gestation. The plasma MOTS-c levels were tested using an ELISA kit (Cloud-Clone Corp., Wuhan, China) according to manufacturer's recommended protocol.

2.2. Animal model and study design

C57BL/6J mice (8 weeks old) were purchased from the animal core facility of Nanjing Medical University. Mice were housed in a stable facility with 12 h light/12 h dark cycles at 25 °C. All mice were stabilized for a week before initiating any experimental procedures. A GDM mouse model was established by feeding mice with a high-fat diet (HFD; 60% kcal) for 4 weeks before pregnancy and was maintained with HFD until delivery. Normal pregnant (NP) mice were fed a normal chow diet throughout pregnancy. Female mice were mated with male mice overnight at a proportion of 2:1 per cage. Pregnancy was determined by the presence of vaginal plugs the next morning, which was identified as gestational day 0. The next day, the mice in GDM group were intraperitoneally injected with STZ (30 mg/kg, dissolved in 0.1 mmol/L citrate buffer, pH 4.2–4.5), followed by 3 injections every 24 h. The NP mice were injected with a similar amount of citrate buffer. Random blood glucose was tested from the tail by glucometer (Roche, USA) 72 h after the first STZ injection. Mice with random blood glucose $\geq$ 11.1 mmol/L were regarded as qualified GDM mice [18]. The GDM mice were then randomly divided into two groups: the GDM group and the MOTS-c group. NP mice were served as a normal control. The MOTS-c group was intraperitoneally injected with MOTS-c (10 mg/kg, dissolved in ddH_2O_2 per day). The GDM and NP groups were injected with the same dose of ddH_2O_2 daily. On gestational day 18, some mice were sacrificed using CO_2 gas, and the skeletal muscle, pancreas and other tissues were collected. Otherwise, the remaining mice gave birth naturally to assess the outcomes in offspring. All the animal experiments conformed to guidelines for the Care and Use of Animals published by Institutional Animal Ethical Committee.

2.3. Glucose tolerance and insulin tolerance tests

For the glucose tolerance test (GTT), mice were fasted 16 h and then intraperitoneally injected with 1.5 g/kg body weight of glucose dissolved in saline. Blood glucose levels were then measured from tail vein blood with a glucometer and test strips at 0, 15, 30, 60, 90, and 120 min after injection. For the insulin tolerance test (ITT), the experimental mice were intraperitoneally injected with insulin (0.5 U/kg) after a 6-h fast, and the blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min after injection. The results of GTTs and ITTs were displayed as blood glucose curves and the area under the curve (AUC).

2.4. Insulin and adiponectin levels measurement

Blood (0.1–0.2 ml) was obtained from an incision of the tail vein using a capillary tube after 16-h fasting at gestational day 18. Both the insulin and adiponectin levels of mice were tested using ELLISA kits (CUSABIO, China).

2.5. In vivo bioluminescence and imaging

The tissue distribution of MOTS-c in vivo was observed using bioluminescence imaging. Briefly, fluorescein isothiocyanate (FITC)-labeled MOTS-c was injected into mice at a dose of 2 mg per mouse. The mice were then anesthetized and major tissues including the heart, liver, spleen, kidney, intestine, pancreases and muscle were excised and imaged using an IVIS spectrum imaging system (PerkinElmer) 3 h after injection. Bioluminescence images were analyzed using Living Image software 4.1.

2.6. C2C12 cell culture and glucose uptake assay

C2C12 myoblasts were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% Penicillin/ Streptomycin (P/S) at 37 °C in humidified air containing 5% CO_2. When the myoblasts were confluent, differentiation inducers (DMEM containing 2% horse serum) were added and the cells were maintained for 5 days. Then, myotubes were stimulated with 0.4 mM palmitate (PA, Sigma-Aldrich) for 24 h to induce insulin resistance. Glucose uptake detection was performed as previous reported [19]. In brief, confluent myotubes were incubated with MOTS-c (10 μM) for 24 h in glucose free DMEM. The culture medium was removed, and cells were washed 3 times with cold phosphate-buffered saline (PBS). The glucose uptake experiment was performed according to the kit protocol (Promega, #J1341, USA). Luminescence signals were recorded with 0.3–1-second integration on a

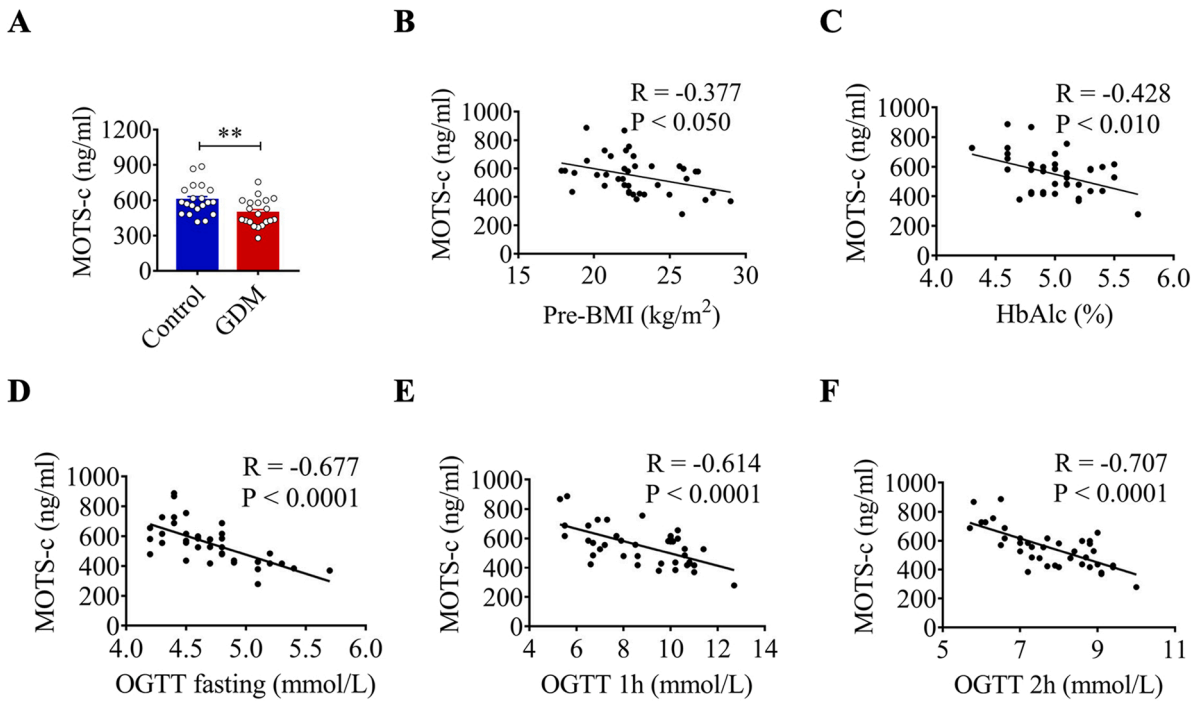

Fig. 1. Circulating MOTS-c levels are decreased in women with GDM. (A) Expression levels of circulating MOTS-c in women with GDM and healthy controls (n = 20). Error bar represents SEM. **p < 0.01 versus control group, two-tailed unpaired *t*-test. (B-F) Correlation coefficient between the plasma MOTS-c levels and pre-pregnancy BMI (B), HbAlc (%) at OGTT (C), blood glucose of OGTT fasting (D), OGTT 1 h (E) and OGTT 2 h (F) in pregnant women. Relationships were evaluated by Pearson's correlation. OGTT, oral glucose tolerance test.

luminometer.

2.7. Western blot analysis

Protein was extracted from skeletal muscle and C2C12 myotubes using RIPA buffer (Beyotime Biotechnology, Beijing, China) containing protease inhibitors (Roche, San Diego, CA, USA) and phosphorylase inhibitors (Roche, San Diego, CA, USA). For Western blot analysis, an equal amount of protein sample was loaded in each well of a 4–12% gradient SDS-PAGE gel. After electrophoresis, proteins were transferred to PVDF membranes, blocked for 2 h with 5% BSA and then incubated with primary antibodies at 4 °C overnight. Antibodies against P-AMPKα (#2535, 1:1000), AMPK (#5831, 1:1000), P-AKT (#4060, 1:1000), AKT (#4685, 1:1000), and GLUT4 (#2213, 1:1000) were purchased from Cell Signaling Technology (CST). Then, the membranes were incubated with HRP-conjugated secondary antibodies for 1 h after washing with TBST, and then developed with an ECL kit. Immunoreactive bands were quantified by densitometric analysis using ImageJ.

2.8. Hematoxylin and eosin staining

Pancreas tissues were immediately fixed with 4% paraformaldehyde, embedded in paraffin and cut into 5-μm-thick sections. The sections were then stained with hematoxylin and eosin (H&E). Briefly, sections were stained in hematoxylin dye for 3–5 min. After washing, sections were dehydrated in alcohol successively for 5 min, and stained in eosin dye for 5 min. Sections were then sealed and observed with a microscope.

2.9. Immunofluorescence staining

Paraffin-embedded pancreatic sections were deparaffinized using Antigen Retrieval Buffer (ab93678, Abcam) following the suggested protocol. Sections were then blocked with 5% normal donkey serum in 1 × PBS at room temperature for 60–90 min in a humidified chamber. Primary antibodies against insulin (CST #8138, 1:200), glucagon (CST #2760S, 1:200) and Ki67 (Proteintech #27309–1-AP, 1:200) diluted in 1% BSA were added and slides were then incubated overnight at 4 °C. After washing with PBS, secondary antibodies (Alexa Fluor 488/594; Invitrogen, USA) were applied to these slides and they were then incubated for 1 h at 37 °C. Then, slides were rinsed and counterstained with DAPI. Samples were photographed using a confocal microscope (Zeiss, LSM-710).

2.10. Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM). The differences between two groups were analyzed using a two-tailed Student's *t*-test. The analysis was performed using Microsoft Excel and/or GraphPad Prism. P-values < 0.05 were considered statistically significant.

3. Result

3.1. Circulating MOTS-c levels are decreased in women with GDM

To explore the circulating level changes of MOTS-c induced by GDM, we collected 40 blood samples from 20 women with GDM and 20 healthy pregnant women. The basic characteristics of the control and GDM pregnant women are presented in Supplemental Table 1. When compared to healthy controls, women with GDM had significantly higher levels of pre-pregnancy body mass index (Pre-BMI), glycated hemoglobin percentage (HbA1c%) and blood glucose during an OGTT. However, there were no differences in average age, gestational weight gain, gestational age of delivery, and neonatal birth weight between these two groups.

Strikingly, GDM women showed significantly lower levels of plasma MOTS-c than healthy controls (494.56 ± 26.26 vs. 602.6 ± 29.02, respectively; p < 0.01, Fig. 1A). Next, we analyzed the correlations between circulating MOTS-c levels and the basic characteristics of the

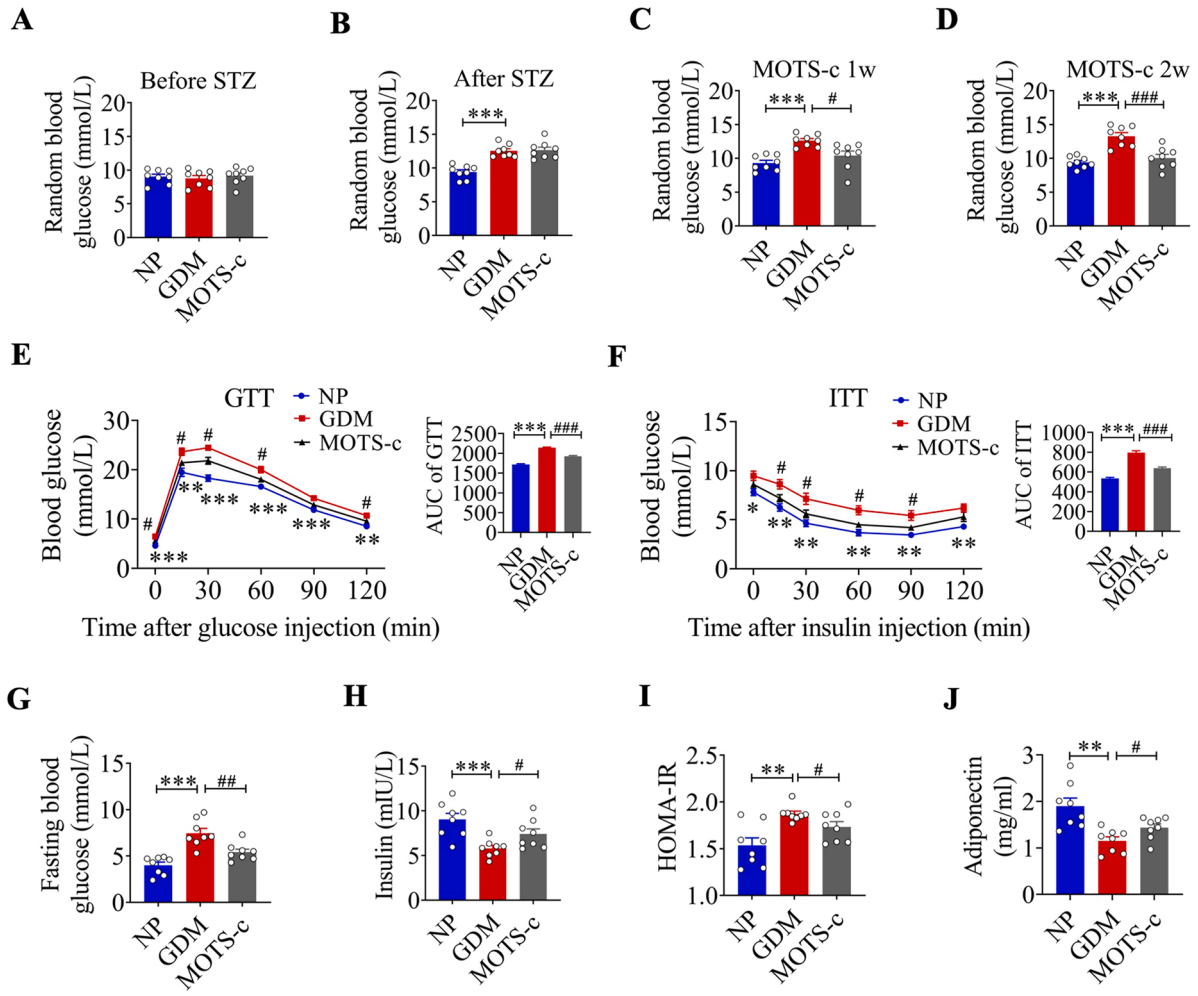

Fig. 2. MOTS-c ameliorates GDM symptoms in a GDM mouse model. (A-B) Random blood glucose levels before (A) and after (B) STZ injection (n = 8). (C-D) Random blood glucose levels after 1-week (C) and 2-week (D) treatment with MOTS-c (n = 8). (E-F) GTT and ITT assays. Mice on gestation day 13–15 were fasted for 16 h or 6 h to perform GTT (1.5 g/kg body weight) and ITT (0.5 U/kg body weight) experiments. (G-J) Fasting blood glucose (G), insulin (H), HOMA-IR (I) and adiponectin (J) levels on gestation day 18. Statistics were performed using the two-tailed Student's *t*-test. *p < 0.05, **p < 0.01, ***p < 0.001, for difference between GDM versus NP group; #p < 0.05, ##p < 0.01, ###p < 0.001, for difference between MOTS-c versus GDM group. NP, normal pregnancy. STZ, streptozotocin.

pregnant women recruited. We found that the plasma MOTS-c concentration was negatively associated with Pre-BMI (R = −0.377, p < 0.05), HbA1c (%) at OGTT (R = −0.428, p < 0.01), and blood glucose at OGTT fasting (R = −0.677, p < 0.0001), OGTT 1 h (R = −0.614, p < 0.0001) and OGTT 2 h (R = −0.707, P < 0.0001) (Fig. 1B-F). These results suggest that MOTS-c may play a role in regulating glucose homeostasis and the development of GDM.

3.2. MOTS-c ameliorates GDM symptoms in a GDM mouse model

To test whether MOTS-c had any therapeutic effect on GDM, we constructed a GDM mouse model by HFD combined with multiple injections of low doses of STZ. We treated GDM mice with MOTS-c (10 mg/kg) daily via intraperitoneal injection. The random blood glucose changes before and after injection of STZ suggested we had successfully established a GDM mouse model (Fig. 2A-B). Random blood glucose decreased significantly after one week of treatment with MOTS-c (Fig. 2C). After 2 weeks of administration, the GDM group's random blood glucose levels continued to rise, while the MOTS-c group showed a further decrease in blood glucose (Fig. 2D).

To further assess the effects of MOTS-c treatment on glucose homeostasis, we subjected mice to a GTT analysis and found a lower basal blood glucose level and enhanced glucose clearance in the MOTS-c group compared with the GDM group (Fig. 2E). Next, our ITT results showed a stronger insulin response in the MOTS-c group (Fig. 2F). We then determined the blood glucose, insulin and adiponectin expression levels in these three groups. Fasting blood glucose was decreased after MOTS-c treatment (Fig. 2G). MOTS-c treatment also increased insulin and adiponectin levels, and improved insulin resistance in GDM mice (Fig. 2H-J). Taken together, the above results showed that MOTS-c could improve insulin resistance and reduce hyperglycemia in GDM mice.

3.3. MOTS-c improves birth outcomes of GDM offspring

To determine the effects of MOTS-c treatment on fetal development, we further observed the birth outcomes of these three groups. Although birth number was similar between the three groups (Fig. 3A), the death number was significantly reduced in the MOTS-c group compared with GDM model controls (Fig. 3B). Furthermore, the birth weight of both male and female offspring was decreased after MOTS-c treatment (Fig. 3C-D). Therefore, we next continued to monitor the weight changes from weaning to adulthood (8 weeks old). However, the differences in body weight between GDM and MOTS-c offspring gradually disappeared from 4 weeks of age (Fig. 3E-F). No significant differences were observed in the fasting blood glucose levels and GTT and ITT results both in male and female offspring between the three groups (Fig. 3G-L). These data show that MOTS-c may have a protective effect on GDM offspring from the intrauterine growth stage of pregnancy to the early period after

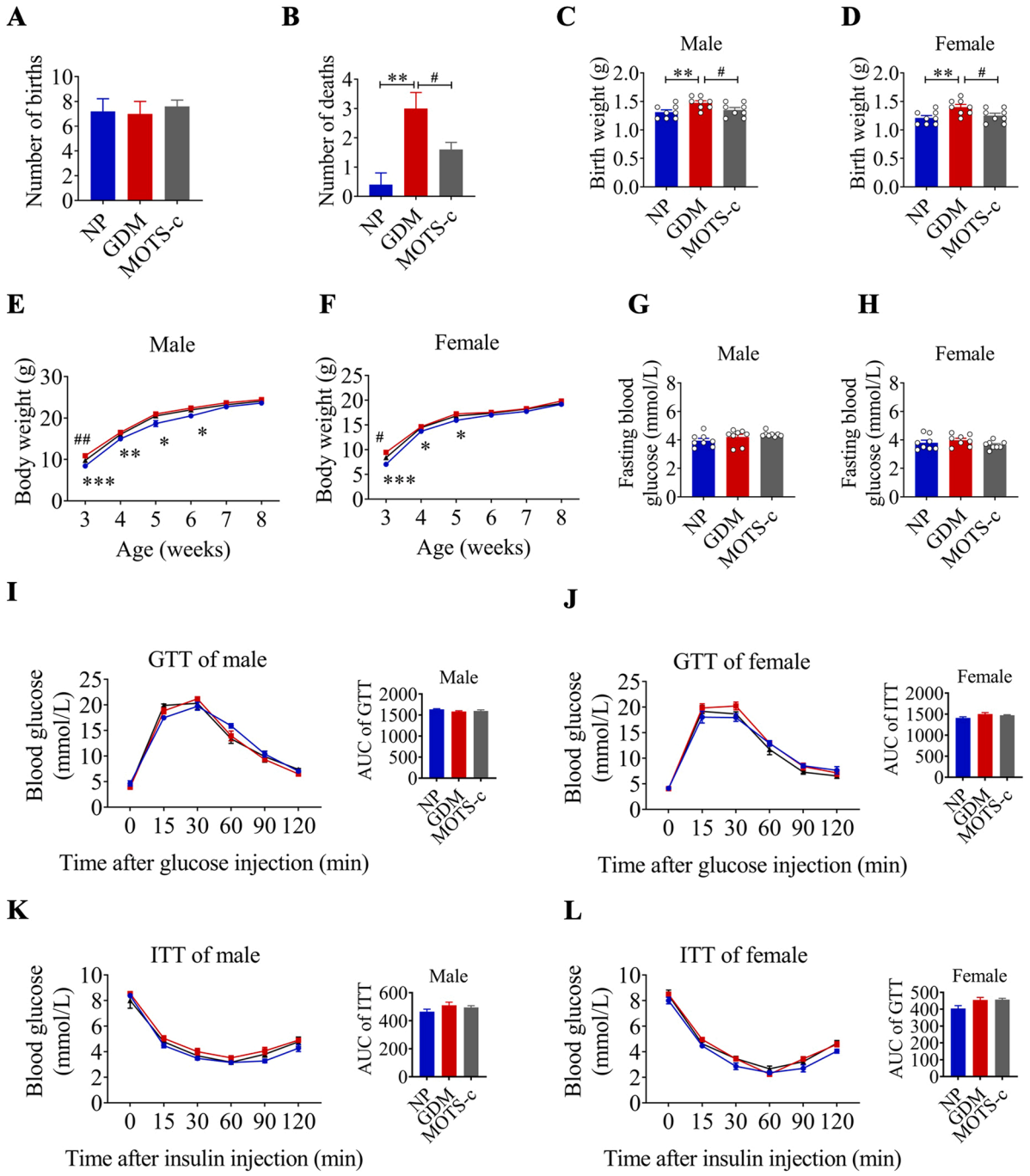

Fig. 3. MOTS-c improves birth outcomes of GDM offspring. (A-B) Born and death number of NP, GDM and MOTS-c offspring (n = 16). (C-D) Birth weight of male and female mice (n = 8). (E-F) Body weight of male and female mice from weaning to the 8th week. (G-H) Fasting blood glucose of male (G) and female (H) mice. (I-J) GTT and ITT assays. Mice of 8 weeks were fasted for 16 h or 6 h to perform GTT (1.5 g/kg body weight) and ITT (0.5 U/kg body weight) experiments. Statistics were performed using the two-tailed Student's *t*-test. *p < 0.05, **p < 0.01, ***p < 0.001 versus NP. #p < 0.05, ##p < 0.01, ###p < 0.001 versus GDM.

birth, which is very important for the development that occurs in later life.

3.4. MOTS-c targets skeletal muscle and enhances insulin sensitivity

Using the bioluminescence imaging technique, we found that MOTS-c mainly accumulated in the pancreas and intestine, and to a lesser extent, in skeletal muscle, the liver, kidney and spleen (Fig. 4A). No fluorescence signal was observed in the organs of mice that received PBS only. Previous reports showed that MOTS-c could target skeletal muscle and lead to the activation of AMPK signaling. We thus measured the changes in the AMPK/AKT signaling pathway in GDM mice treated with MOTS-c. Mice were sacrificed at the 18th day of gestation and protein was extracted from skeletal muscles for Western blot. As indicated in Fig. 4B, MOTS-c treatment also increased the phosphorylation of AMPK and AKT and the expression level of GLUT4 in skeletal muscle. We next examined the effect of MOTS-c on glucose disposal in vitro. Following differentiation to mature myotubes, C2C12 myoblasts were treated with PA to induce insulin resistance. The presence of MOTS-c could attenuate PA-induced insulin resistance, as indicated by the elevated phosphorylation of AMPK and AKT, as well as GLUT4 expression (Fig. 4C). Moreover, we found that MOTS-c significantly increased the basal

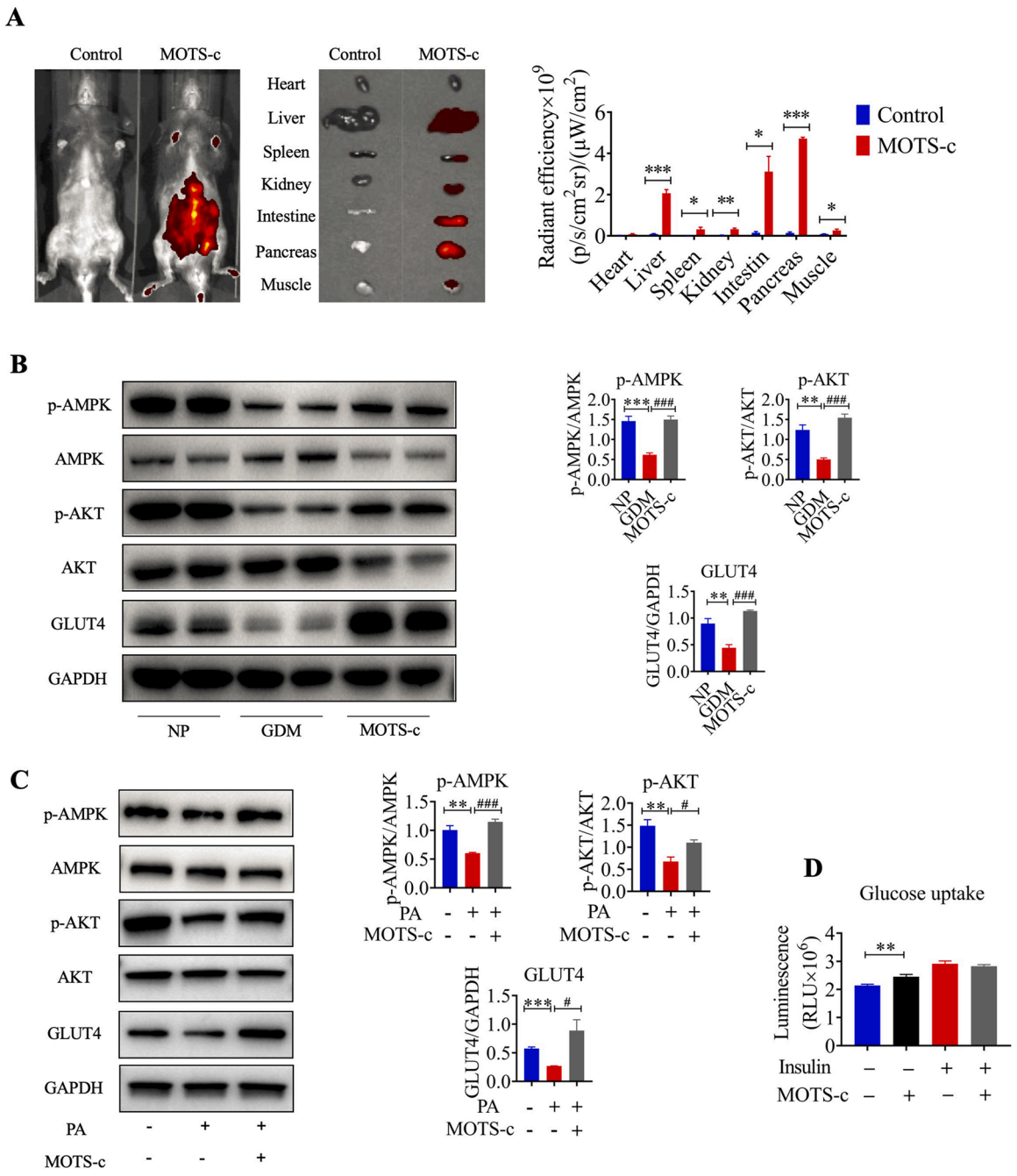

Fig. 4. MOTS-c targets skeletal muscle and enhances insulin sensitivity. (A) Tissue distribution of FITC-labeled MOTS-c (left panel) and fluorescent intensity analysis (right panel). (B-C) Western blot analysis were performed to determine the phosphorylation of AMPK and AKT and GLUT4 expression in skeletal muscle from GDM mice or C2C12 myotubes with or without MOTS-c treatment. (D) Impact of MOTS-c on glucose uptake of C2C12 myotubes. Data were presented as mean ± SEM. Statistics were performed using the two-tailed Student's *t*-test. *p < 0.05, **p < 0.01, ***p < 0.001 versus NP. #p < 0.05, ##p < 0.01, ###p < 0.001 versus GDM. Ctrl, Control; PA, palmitate.

glucose uptake levels of C2C12 myotubes, although there was no statistical difference when stimulated with insulin (Fig. 4D).

3.5. MOTS-c protects pancreatic β-cells from STZ-induced injury

STZ challenge can lead to selective pancreatic β-cell apoptosis and result in impaired insulin secretion and increased blood glucose levels [20]. When doing the histological analyses of pancreases, we observed that the islet number and volume were dramatically reduced in GDM mice after STZ administration, while MOTS-c treatment partially restored these morphology changes (Fig. 5A-B). Moreover, compared to mice treated with STZ only, mice treated with MOTS-c showed a remarkable increase in the number of insulin-positive and the total mass of β-cells in islets (Fig. 5C-D). It is well-known that increased β-cell proliferation is the main underlying mechanism for islet expansion [21]. We thus analyzed the proliferation of pancreatic β-cells in these groups. Immunofluorescence showed that the positivity rate for the proliferation marker Ki67 protein in pancreatic β cells from mice treated with MOTS-c was significantly increased compared with control mice (Fig. 5E-F). These results implied that MOTS-c exerted a protective effect against the β-cell injury induced by STZ.

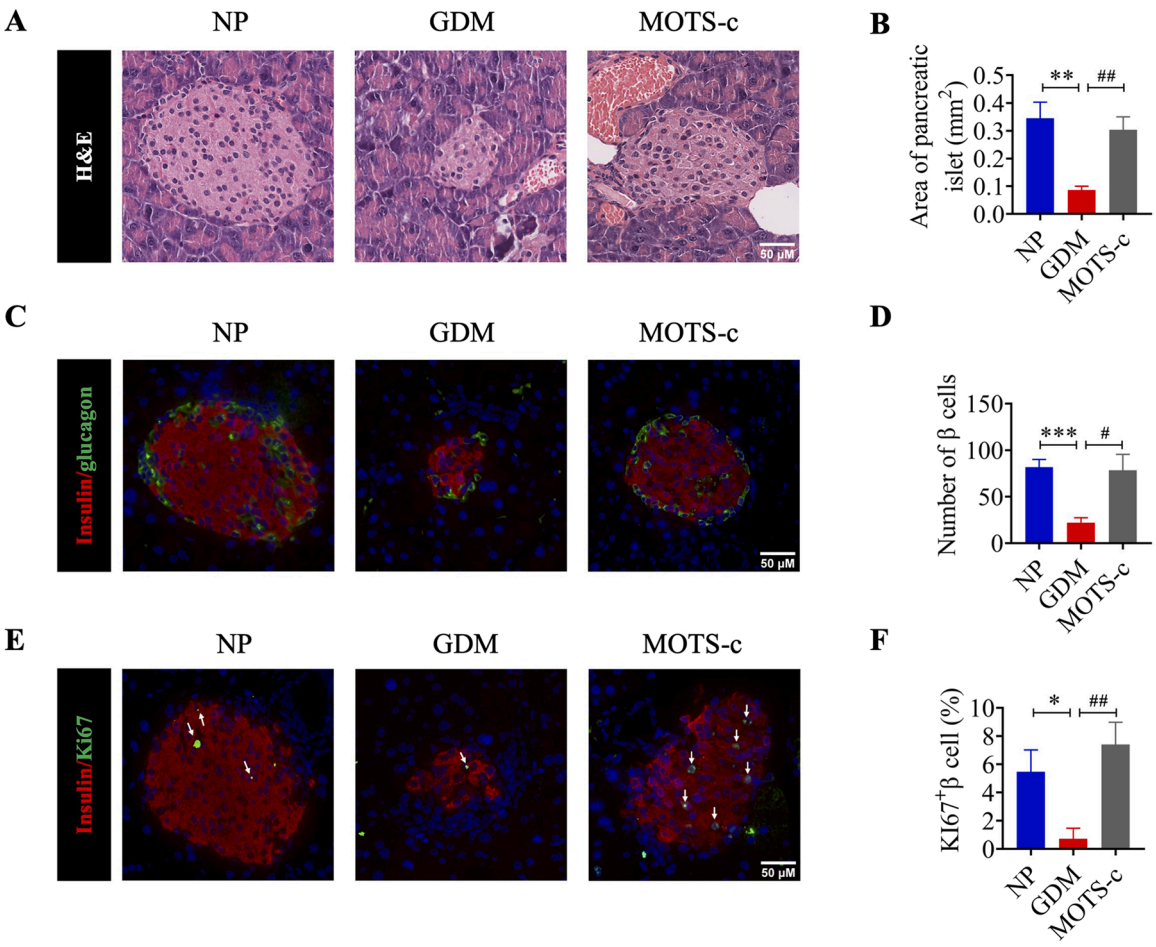

Fig. 5. MOTS-c protects pancreatic β-cell from STZ-induced injure. H&E staining (A) and area analysis (B) of islets histology sections from NP and GDM mice models with or without MOTS-c treatment (n = 4). Analysis of insulin-positive β cells (C-D) and Ki67-positive β cells ratio (%) (E-F) by immunofluorescence staining of pancreatic islets (n = 4). Data were presented as mean ± SEM. Statistics were performed using the two-tailed Student's *t*-test. *p < 0.05, **p < 0.01, ***p < 0.001 versus NP. #p < 0.05, ##p < 0.01, ###p < 0.001 versus GDM. NP, normal pregnancy.

4. Discussion

Over the past few decades, changes in lifestyle and diet have resulted in increased obesity and other risk factors and has thus accelerated the prevalence of GDM [22]. Given that the available strategies for GDM treatment are limited, searching for a safe and effective drug is especially urgent. In present study, we uncovered the expression changes of MOTS-c in women with GDM and demonstrated that administration of MOTS-c could improve insulin resistance and glucose intolerance, leading to attenuation of the adverse productive outcomes in GDM. Mechanistically, MOTS-c sensitizes insulin signaling through AMPK in skeletal muscle and protects the β cells from STZ-induced damage. The insulin resistance induced by pregnancy and following inadequate insulin secretion compensating for increased insulin requirements are the main causes of GDM. MOTS-c could be a potential therapeutic tool for the treatment of GDM. To the best of our knowledge, this is the first report to explore the circulating levels of MOTS-c in women with GDM and assess its therapeutic effects in GDM mouse model.

The insulin resistance induced by hormones and adipokines secreted from the placenta during pregnancy is the root cause of GDM [2,8]. Specifically, maternal insulin resistance is the key feature of pregnancies complicated by GDM, which also leads to increased fetal nutrient supply and fetal adiposity. It has been reported that the insulin sensitivity reduces approximately 50% in those with GDM when compared with normal pregnancy [23]. Thus, improving the sensitivity of insulin-targeted organs can be easier to achieve than other treatments to combat GDM. The skeletal muscle, adipose tissue and liver are the main organs that act in response to insulin signaling to ensure homeostatic control of blood glucose levels [24]. Here, we revealed that MOTS-c improved insulin resistance in skeletal muscle from mice with GDM, which was indicated by the activation of AMPK signaling. AMPK serves as a crucial cellular energy sensor that promote glucose transport and fatty acid oxidation in skeletal muscle [25]. In vitro studies showed that MOTS-c treatment increased basal glucose transport, whereas insulin-stimulated uptake was unaffected. Previous studies report that insulin diminishes AMPK activity in myotubes though stimulating phosphorylation of $Ser^{485/491}$ AMPK, an inhibitory phosphorylation site of AMPK [26]. This may help to explain why MOTS-c failed to enhance glucose uptake in the presence of insulin in vitro. Moreover, increasing evidence shows that AMPK is dysregulated in animals and humans with GDM [27]. Thus, it is reasonable to propose that MOTS-c functions at least partly though targeting skeletal muscle to improve insulin resistance in mice with GDM.

To compensate for the increased insulin demands due to peripheral insulin resistance, material islets undergo expansion, i.e., hypertrophy, proliferation and neogenesis [28]. The development of GDM occurs when a woman's pancreas does not secrete enough insulin to keep up with the metabolic stress of insulin resistance [8]. Here, we report that the insulin levels were elevated in GDM mice treated with MOTS-c. Further results showed that MOTS-c cold provide protection against the β-cell destruction induced by STZ and increase the proliferation of β-cells. This may account for the increased insulin levels after MOTS-c

administration and also provide another factor to illustrate the therapeutic effects of MOTS-c in combatting GDM. Another recent study also revealed that MOTS-c could prevent pancreatic β-cell destruction in a non-obese diabetic mouse model of T1D by reducing T cell activation [17]. It was reported that pancreatic islet inflammatory lesions are an important cause of STZ-induced diabetes mellitus [29]. MOTS-c has been shown to have the ability to inhibit inflammation in several studies [30–32], which highlights new ways to investigate the mechanism of MOTS-c.

Poor productive outcomes, including macrosomia, high death rates of offspring, birth injury and neonatal hypoglycemia, are other major adverse symptoms of GDM [5]. In general, the offspring of GDM mothers are prone to be large for their gestational age or exhibit macrosomia. Maternal hyperglycemia leads to increased placental glucose transport, stimulating the production of endogenous fetal insulin and insulin-like growth factor 1 (IGF-1), which together lead to fetal overgrowth and results in a higher incidence rate of fetal macrosomia [33,34]. This increases the risk of perinatal death for infants and mothers with GDM. Improved offspring number and body weight were observed in our GDM mice model treated with MOTS-c. However, these differences in body weight gradually disappeared as the mice grew older. Therefore, we hypothesize that the improvement in birth outcome in the MOTS-c group were mainly due to the therapeutic effect on maternal GDM, and this effect gradually disappeared after delivery and separation from the maternal environment. Offspring born to mothers with GDM also had an increased likelihood of developing obesity, T2DM and associated metabolic diseases when compared with nondiabetic mothers later in life [35,36]. Thus, extended monitoring of offspring from GDM mice may help further an understanding of the protective effect of MOTS-c.

5. Conclusion

In conclusion, MOTS-c administration significantly alleviates hyperglycemia and improves insulin resistance in GDM mice, and results in improved fetal development and reproductive outcomes. Further analysis revealed that these effects of MOTS-c were mediated by activating glucose disposal in skeletal muscle and protecting against STZ-induced β-cell destruction. However, there were still some limitations of the current study. First, an additional GDM animal model (e.g., *db/+* mice) would have made the present results more convincing. Second, more extended studies should focus on the safety and efficacy of MOTS-c in the future.

Ethics statement

All the procedures were approved by the Ethics Committee of Nanjing Maternal and Child Health Hospital (Permission Number 2018KY-007), and written informed consent was signed by all adult participants. The animal experiments were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University (Approval Number IACUC-2011052).

CRediT authorship contribution statement

Yadong Yin, Yihui Pan, Jin He and Yangyang Wu designed and performed the experiments and interpreted the results of experiments. Yadong Yin, Lan Liu and Xianwei Cui designed, conceived, supervised the study, and written the manuscript. Hong Zhong provided material support and assisted in writing of the manuscript. Xianwei Cui and Chenbo Ji provided the funding and approved the final version of the manuscript.

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Declaration of competing interest

The authors have declared that no conflict of interest exists.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2021.105987.

References

[1] E.C. Johns, F.C. Denison, J.E. Norman, R.M. Reynolds, Gestational diabetes mellitus: mechanisms, treatment, and complications, Trends Endocrinol. Metab. 29 (11) (2018) 743–754.
[2] S.R. Murray, R.M. Reynolds, Short- and long-term outcomes of gestational diabetes and its treatment on fetal development, Prenat. Diagn. 40 (9) (2020) 1085–1091.
[3] H. Melchior, D. Kurch-Bek, M. Mund, The prevalence of gestational diabetes, Dtsch Arztebl Int. 114 (24) (2017) 412–418.
[4] J. Juan, H. Yang, Prevalence, prevention, and lifestyle intervention of gestational diabetes mellitus in china, Int. J. Environ. Res Public Health 17 (24) (2020).
[5] L.J. England, P.M. Dietz, T. Njoroge, W.M. Callaghan, C. Bruce, R.M. Buus, D. F. Williamson, Preventing type 2 diabetes: public health implications for women with a history of gestational diabetes mellitus, Am. J. Obstet. Gynecol. 200 (4) (2009) 361–365.
[6] C. Kim, K.M. Newton, R.H. Knopp, Gestational diabetes and the incidence of type 2 diabetes: a systematic review, Diabetes Care 25 (10) (2002) 1862–1868.
[7] S. Rieck, K.H. Kaestner, Expansion of beta-cell mass in response to pregnancy, Trends Endocrinol. Metab. 21 (3) (2010) 151–158.
[8] J.F. Plows, J.L. Stanley, P.N. Baker, C.M. Reynolds, M.H. Vickers, The pathophysiology of gestational diabetes mellitus, Int. J. Mol. Sci. 19 (11) (2018).
[9] M. Subiabre, L. Silva, F. Toledo, M. Paublo, M.A. Lopez, M.P. Boric, L. Sobrevia, Insulin therapy and its consequences for the mother, foetus, and newborn in gestational diabetes mellitus, Biochim. Biophys. Acta Mol. Basis Dis. 1864 (9 Pt B) (2018) 2949–2956.
[10] H. Zhu, B. Chen, Y. Cheng, Y. Zhou, Y.S. Yan, Q. Luo, Y. Jiang, J.Z. Sheng, G. L. Ding, H.F. Huang, Insulin therapy for gestational diabetes mellitus does not fully protect offspring from diet-induced metabolic disorders, Diabetes 68 (4) (2019) 696–708.
[11] C. Lee, J. Zeng, B.G. Drew, T. Sallam, A. Martin-Montalvo, J. Wan, S.J. Kim, H. Mehta, A.L. Hevener, R. de Cabo, P. Cohen, The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance, Cell Metab. 21 (3) (2015) 443–454.
[12] C. Lee, K.H. Kim, P. Cohen, MOTS-c: a novel mitochondrial-derived peptide regulating muscle and fat metabolism, Free Radic. Biol. Med. 100 (2016) 182–187.
[13] C. Du, C. Zhang, W. Wu, Y. Liang, A. Wang, S. Wu, Y. Zhao, L. Hou, Q. Ning, X. Luo, Circulating MOTS-c levels are decreased in obese male children and adolescents and associated with insulin resistance, Pedia Diabetes (2018).
[14] L.R. Cataldo, R. Fernandez-Verdejo, J.L. Santos, J.E. Galgani, Plasma MOTS-c levels are associated with insulin sensitivity in lean but not in obese individuals, J. Invest. Med. 66 (6) (2018) 1019–1022.
[15] M. Ramanjaneya, I. Bettahi, J. Jerobin, P. Chandra, K.C. Abi, M. Skarulis, S. L. Atkin, A.B. Abou-Samra, Mitochondrial-Derived peptides are down regulated in diabetes subjects, Front. Endocrinol. 10 (2019) 331.
[16] J.C. Reynolds, R.W. Lai, J. Woodhead, J.H. Joly, C.J. Mitchell, D. Cameron-Smith, R. Lu, P. Cohen, N.A. Graham, B.A. Benayoun, T.L. Merry, C. Lee, MOTS-c is an exercise-induced mitochondrial-encoded regulator of age-dependent physical decline and muscle homeostasis, Nat. Commun. 12 (1) (2021) 470.
[17] B.S. Kong, S.H. Min, C. Lee, Y.M. Cho, Mitochondrial-encoded MOTS-c prevents pancreatic islet destruction in autoimmune diabetes, Cell Rep. 36 (4) (2021), 109447.
[18] Y. He, N. Wu, W. Yu, L. Li, H. Ouyang, X. Liu, M. Qian, A. Al-Mureish, Research progress on the experimental animal model of gestational diabetes mellitus, Diabetes Metab. Syndr. Obes. 13 (2020) 4235–4247.
[19] X. Cui, L. You, L. Zhu, X. Wang, Y. Zhou, Y. Li, J. Wen, Y. Xia, X. Wang, C. Ji, X. Guo, Change in circulating microRNA profile of obese children indicates future risk of adult diabetes, Metabolism 78 (2018) 95–105.
[20] D.C. Damasceno, A.O. Netto, I.L. Iessi, F.Q. Gallego, S.B. Corvino, B. Dallaqua, Y. K. Sinzato, A. Bueno, I.M. Calderon, M.V. Rudge, Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes, Biomed. Res. Int. 2014 (2014), 819065.
[21] S. Georgia, A. Bhushan, Beta cell replication is the primary mechanism for maintaining postnatal beta cell mass, J. Clin. Invest. 114 (7) (2004) 963–968.
[22] X.F. Pan, L. Wang, A. Pan, Epidemiology and determinants of obesity in China, Lancet Diabetes Endocrinol. 9 (6) (2021) 373–392.
[23] P.M. Catalano, Trying to understand gestational diabetes, Diabet. Med. 31 (3) (2014) 273–281.

[24] A. Chadt, H. Al-Hasani, Glucose transporters in adipose tissue, liver, and skeletal muscle in metabolic health and disease, Pflug. Arch. 472 (9) (2020) 1273–1298.
[25] A. Gonzalez, M.N. Hall, S.C. Lin, D.G. Hardie, AMPK and TOR: the yin and yang of cellular nutrient sensing and growth control, Cell Metab. 31 (3) (2020) 472–492.
[26] R.J. Valentine, K.A. Coughlan, N.B. Ruderman, A.K. Saha, Insulin inhibits AMPK activity and phosphorylates AMPK Ser485/491 through Akt in hepatocytes, myotubes and incubated rat skeletal muscle, Arch. Biochem. Biophys. 562 (2014) 62–69.
[27] K.A. Coughlan, R.J. Valentine, N.B. Ruderman, A.K. Saha, AMPK activation: a therapeutic target for type 2 diabetes? Diabetes Metab. Syndr. Obes. 7 (2014) 241–253.
[28] S. Ernst, C. Demirci, S. Valle, S. Velazquez-Garcia, A. Garcia-Ocana, Mechanisms in the adaptation of maternal beta-cells during pregnancy, Diabetes Manag. 1 (2) (2011) 239–248.
[29] D.C. Damasceno, A.O. Netto, I.L. Iessi, F.Q. Gallego, S.B. Corvino, B. Dallaqua, Y. K. Sinzato, A. Bueno, I.M. Calderon, M.V. Rudge, Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes, Biomed. Res. Int. 2014 (2014), 819065.
[30] Z. Yan, S. Zhu, H. Wang, L. Wang, T. Du, Z. Ye, D. Zhai, Z. Zhu, X. Tian, Z. Lu, X. Cao, , MOTS-c inhibits Osteolysis in the Mouse Calvaria by affecting osteocyte-osteoclast crosstalk and inhibiting inflammation, Pharm. Res. 147 (2019), 104381.
[31] J. Jiang, X. Chang, Y. Nie, Y. Shen, X. Liang, Y. Peng, M. Chang, Peripheral administration of a Cell-Penetrating MOTS-c analogue enhances memory and attenuates abeta1-42- or LPS-Induced memory impairment through inhibiting neuroinflammation, ACS Chem. Neurosci. 12 (9) (2021) 1506–1518.
[32] D. Zhai, Z. Ye, Y. Jiang, C. Xu, B. Ruan, Y. Yang, X. Lei, A. Xiang, H. Lu, Z. Zhu, Z. Yan, D. Wei, Q. Li, L. Wang, Z. Lu, MOTS-c peptide increases survival and decreases bacterial load in mice infected with MRSA, Mol. Immunol. 92 (2017) 151–160.
[33] A. Ericsson, K. Saljo, E. Sjostrand, N. Jansson, P.D. Prasad, T.L. Powell, T. Jansson, Brief hyperglycaemia in the early pregnant rat increases fetal weight at term by stimulating placental growth and affecting placental nutrient transport, J. Physiol. 581 (Pt 3) (2007) 1323–1332.
[34] G. Desoye, M.S. Hauguel-De, The human placenta in gestational diabetes mellitus. The insulin and cytokine network, Diabetes Care 30 (Suppl 2) (2007) S120–S126.
[35] W.H. Tam, R. Ma, R. Ozaki, A.M. Li, M. Chan, L.Y. Yuen, T. Lao, X. Yang, C.S. Ho, G.E. Tutino, J. Chan, In utero exposure to maternal hyperglycemia increases childhood cardiometabolic risk in offspring, Diabetes Care 40 (5) (2017) 679–686.
[36] S.C. Lee, Y.B. Pu, C.C. Chow, V.T. Yeung, G.T. Ko, W.Y. So, J.K. Li, W.B. Chan, R. C. Ma, J.A. Critchley, C.S. Cockram, J.C. Chan, Diabetes in Hong Kong Chinese: evidence for familial clustering and parental effects, Diabetes Care 23 (9) (2000) 1365–1368.