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Research Article

Long-term administration of the α-amylase inhibitor acarbose effective against type 2 diabetes symptoms in C57BL/6 mice

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Abstract

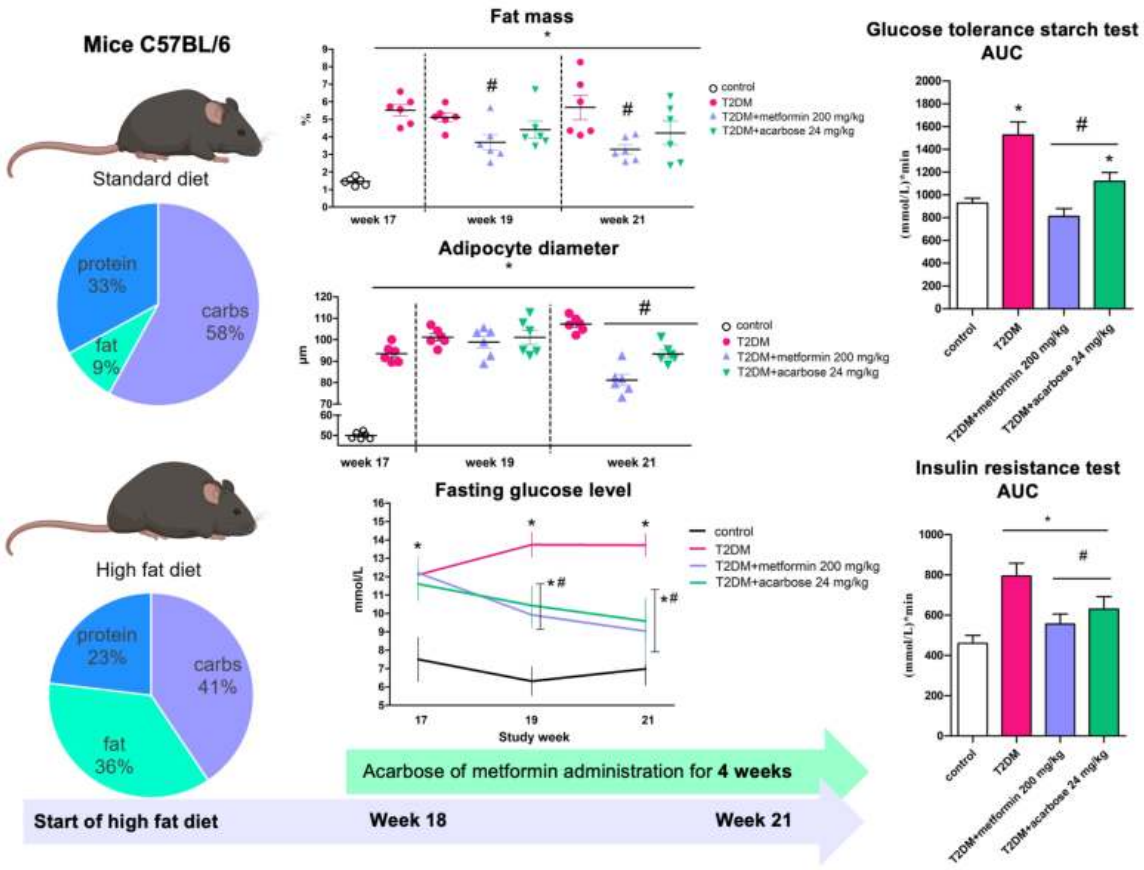
Introduction: α-amylase inhibitors are an important class of second-line antihyperglycemic drugs. They slow down the breakdown and absorption of carbohydrates, reducing peak glucose concentration with meals. Recent reports have also shown other beneficial effects of α-amylase inhibitors on type 2 diabetes mellitus (T2DM).

Material and Methods: T2DM was modeled by keeping C57BL/6 mice on a high-fat diet for 21 weeks. Starting at week 18, the animals were orally administered acarbose at a dose of 24 mg/kg or the comparative drug metformin at a dose of 200 mg/kg for 4 weeks. Body weight gain, visceral fat mass, and adipocyte diameter were monitored during the period of test substances administration. At weeks 17, 19 and 21 of the study, glucose tolerance starch test and insulin resistance test were performed, and fasting blood glucose was measured.

Results: Administration of acarbose for 2 and 4 weeks resulted in a significant reduction of postprandial glucose concentration in the starch test; glucose AUC was significantly lower after administration of acarbose at a dose of 24 mg/kg on the background of T2DM modeling. Acarbose at a dose of 24 mg/kg effectively reduced fasting glucose concentration after 2 and 4 weeks of daily treatment on par with metformin. Administration of acarbose at a dose of 24 mg/kg for 2 and 4 weeks resulted in a significant decrease in the glucose AUC in the insulin resistance test. Acarbose promoted a significant decrease in adipocyte diameter and body weight gain against the background of T2DM modeling.

Conclusion: Long-term acarbose administration at a daily dose of 24 mg/kg is effective in reducing postprandial glucose concentration in mice with T2DM due to its α-amylase inhibitory activity. Additionally, it can alleviate insulin resistance, lower fasting glucose concentration, and prevent obesity development by stimulating GLP-1 secretion.

Graphical abstract



Keywords

acarbose, α-amylase inhibitors, insulin resistance, type 2 diabetes mellitus, C57BL/6 mice

Introduction

Metformin is commonly used as a first-line drug in the treatment of type 2 diabetes mellitus (T2DM). It reduces insulin resistance by inducing GLUT4 translocation (Lee et al. 2012) and also has a significant antihyperglycemic effect, partly by increasing PGC-1α expression through upstream AMPK kinase (Zamanian et al. 2023). However, metformin frequently causes gastrointestinal side effects and is contraindicated in renal dysfunction. It is more commonly used in combination with second-line hypoglycemic agents to achieve better glycemic control when metformin dose reduction is necessary (Petersons 2018).

α-Amylase inhibitors are an important class of second-line antihyperglycemic drugs. They slow down the breakdown and absorption of carbohydrates by inhibiting α-amylase, which hydrolyzes α-D-(1,4)-glycosidic bonds in starch or polysaccharides to monosaccharides. These monosaccharides are then absorbed into the portal vein of the liver through the small intestine (Shestakova 2017; Taslimi et al. 2018).

Acarbose is an α-amylase inhibitor that has a structural similarity to natural oligosaccharides. It has a much higher affinity for α-amylases, up to 10^4-10^5 times higher. By inhibiting α-amylases, it reduces the formation of monosaccharides, which in turn reduces the amount of insulin required for further metabolism. This leads to a decrease in postprandial blood glucose and insulin levels caused by food intake. As a decrease in blood glucose concentration leads to a significant reduction in insulin synthesis and secretion stimulation, acarbose reduces hyperinsulinemia caused by insulin resistance. Therefore, it exhibits hypoglycemic activity in the stomach without being absorbed into the systemic bloodstream (Rosak and Mertes 2012).

However, it has been suggested that the efficacy of acarbose in T2DM may be manifested indirectly through GLP-1 (Dalsgaard et al. 2021). The administration of α-amylase inhibitors results in slow digestion of carbohydrates and breakdown of oligosaccharides. This, in turn, causes undigested carbohydrates to reach the lower small intestine and stimulate GLP-1 secretion. GLP-1 is a hormone that delays gastric emptying, decreases glucagon secretion, and regulates insulin

secretion, depending on blood glucose concentration (Wong et al. 2008). It is considered one of the most effective drugs for the treatment of T2DM. GLP-1 enhances insulin secretion by pancreatic β-cells and reduces glucagon release from pancreatic α-cells. Additionally, GLP-1 can stimulate pancreatic β-cell proliferation and decelerate the progression of T2DM (Li et al. 2022).

Although acarbose has continued to be part of T2DM treatment guidelines and algorithms, newer therapies have taken the spotlight in the past 10-15 years. However, recent studies have shown additional benefits of acarbose in T2DM therapy. It has been found to be effective regardless of age, gender, or body mass index (Altay 2022). Furthermore, α-amylase inhibitors can be combined with all other classes of hypoglycemic drugs (Krasilnikova et al. 2009). Acarbose has been found to reduce the risk of cardiovascular disease in people with T2DM by lowering postprandial blood glucose levels and reducing oxidative stress (Altay 2022). The efficacy of α-amylase inhibitors in T2DM therapy offers multifaceted possibilities beyond correcting postprandial glucose levels with meals.

It is assumed that α-amylase inhibitors reduce fasting blood glucose levels, increase tissue sensitivity to insulin, and prevent obesity on the background of T2DM modeling due to the effect of acarbose on GLP-1 secretion (Dalsgaard et al. 2021).

The study aimed to investigate the efficacy of acarbose monotherapy with repeated administration in an experimental T2DM model.

Materials and Methods

Animals

Ninety adult male C57BL/6 mice aged 4 weeks of SPF-status were obtained from the Pushchino Nursery for Laboratory Animals. The animals underwent an adaptation period of 14 days and were housed in a barrier-type room (barrier zone 2 of Laboratory of Biological Testing of the Branch of the State Scientific Centre Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences (BIBCh RAS) (5 animals each in a Type-3 cage, 845 cm^2). All manipulations with animals were approved by the Institutional Commission for the Control and Use of Laboratory Animals of the BIBCh RAS (Minutes No 767/20 of 23.10.2020). Controlled environmental conditions (temperature 20-24°C, relative humidity 30-70%, with an automatic change of day and night period (08:00-20:00 h - 'day', 20:00-08:00 h - 'night') and at least 12-fold change of room air volume per hour were maintained. The temperature and humidity were automatically monitored in each room using the Eksis Visual Lab software (Praktik NC (Research Center), Russia). Animals without signs of health abnormalities upon clinical examination were selected for the experiment. Animals were distributed into groups using the principle of randomization so that the average body weight of animals by day 1 of the experiment was not statistically different between groups. Each animal was assigned an individual number, according to which the animal was identified by ear punching.

During the experiment, each animal was individually in Type-3 cages (845 cm^2) on bedding for which autoclaved dust-free rodent bedding consisting of wood chips (LIGNOCEL BK8/15, JRS, Germany) was used. Specially prepared water by Milli-RO system (Millipore, USA) was given ad libitum in standard autoclaved drinking bottles with steel caps.

Type 2 diabetes mellitus modeling

Metabolic syndrome in animals was achieved by keeping them on a high-fat diet (HFD) for 21 weeks.

The control group (n=18) received autoclavable standard diet which consisted of a complete ration of granulated rodent food SNIFF RI/M-H V1534-30, ad libitum in the feeding hollow of the cage lid. The total energy value was 306 kcal/100g (58% carbohydrate, 9% fat, 33% protein).

To model T2DM in groups № 2-4 (n=72), the high-fat diet (HFD) was produced using the previously described protocol (Borozdina et al. 2023). For 1 kg of HFD, 610 g of standard rodent diet SNIFF (Ssniff Spezialdiäten GmbH, Germany), 360 g of melted lard (Vkus&Cvet, Russia), ≈250 ml of water (Millipore, USA), 10 g of sodium chloride (Mozyrsalt, Belarus) and 30 g of monosodium glutamate (Baba Klava, Russia) were mixed. The mixture was brewed to the consistency of dough and food granules were formed, which were dried at a temperature of 60-70ºC for 10-12 hours. The prepared HFD was stored at 4ºC for no more than 7 days. The approximate energy value of HFD was 516 kcal/100g (with the content of the main nutrients in the diet at the rate of 41% carbohydrates, 36% fats, 23% proteins).

At week 17 of the experiment, before the administration of the test substances, animals (n=18) with body weight gain of less than 35% relative to week 1 of the experiment were excluded from the groups on HFD. During the test substance administration period, 54 animals with developed T2DM were divided into three groups (№ 2-4), each containing 18 animals with similar average body weights.

Administration of test substances

Starting from the 18th week of the experiment, the animals were orally administered the carrier or test substances daily for 4 weeks.

The carrier (distilled water) was administered orally to groups №1 and №2, in the volume of 5 mL/kg.

Metformin (LLC NPO PharmVILAR, Russia) was administered orally to group № 3, at a dose of 200 mg/kg, in a volume of 5 mL/kg.

Acarbose (Merck, China) was administered orally to group № 4, at a dose of 24 mg/kg, in the volume of 5 mL/kg.

Fasting blood glucose concentration

The animals were fasted for four hours prior to measurement. A drop of blood was obtained by a small incision of the tail tip, and glucose concentration was determined using a Satellite®Express glucometer (ELTA, Russia). Manipulation was performed at 17, 19 and 21 weeks of the study and during the glucose tolerance starch test and insulin resistance test.

Glucose tolerance starch test (GTST)

GTST was performed at 17, 19 and 21 weeks of the study. The animals were fasted for 12 h before the GTST. On the day of GTST, the test substances were administered according to group affiliation 45 min before the test.

Blood glucose concentration was determined after administration of starch (STOING, Russia) solution orally at a dose of 3 g/kg after 0, 30, 60, 90 and 120 minutes. The absolute values of glucose concentration were used to calculate the glucose AUC in GraphPad Prism 5.0 program (USA).

Insulin resistance test

IRT was performed at 17, 19 and 21 weeks of the study. Before the IRT, the animals were fasted for 4 hours. Insulin was administered subcutaneously at a dose of 0.75 IU/kg, in a volume of 5 mL/kg. Blood glucose concentration was measured at 0, 15, 30, 60 and 120 minutes after insulin administration. The absolute values of glucose concentration were used to calculate the glucose AUC in GraphPad Prism 5.0 program (USA).

Body weight gain

Animals were weighed weekly from week 1 to week 21 to monitor body weight gain. At week 17 of the study, animals with a body weight gain of more than 35% relative to week 1 were selected for the experiment. For weeks 18-21 of the study, body weight gain was calculated relative to week 18 of the study.

Visceral fat surrounding epididymis

At 17, 19 and 21 weeks, 6 animals from each group were euthanized. The animal was anesthetized by intramuscular injection with a mixture of Zoletil (Zoetis, Spain) / Xyla (Interchemie werken "De Adelaar" BV, Netherlands). After the onset of the surgical stage of anesthesia, the animal was dissected, the organs were examined for macro-damage, and total blood sampling from the inferior vena cava was performed. Next, the adipose tissue surrounding epididymis was carefully extracted and weighed. Then the adipose tissue was fixed in 10% formalin before staining. After fixing the tissue, it was dehydrated and soaked in paraffin. Sections from the paraffin blocks were stained with hematoxylin and eosin. Histological sections were examined using a Leica DMLA transmitted light microscope(Germany), a Photometrics Cool SNAP cf video camera (USA), and Mekos software (Russia). The diameter of 100 adipocytes in 15-20 random fields of view was measured using ImageJ software (USA) on slices of visceral fat.

Statistical analysis

Descriptive statistics were applied to all quantitative data: mean, standard deviation and standard error of the mean were calculated and presented in the results. To establish intergroup differences, quantitative data were analyzed by Student's t-test for pairwise comparison with control and model group. Statistical analysis was performed using GraphPad Prism 5.0 program (USA). Differences were determined statistically significant at $p<0.05$.

Results

Glucose tolerance starch test

Modeling T2DM in C57BL/6 mice resulted in a significant increase in postprandial glucose levels after starch consumption. The glucose AUC was also significantly elevated by an average of 40% at week 19 and 21 of T2DM modeling.

Acarbose administration for 2 and 4 weeks resulted in significantly lower postprandial glucose concentrations in the GTST (Fig. 1). The glucose AUC was significantly lower after administration of acarbose at a dose of 24 mg/kg on the background of T2DM modeling.

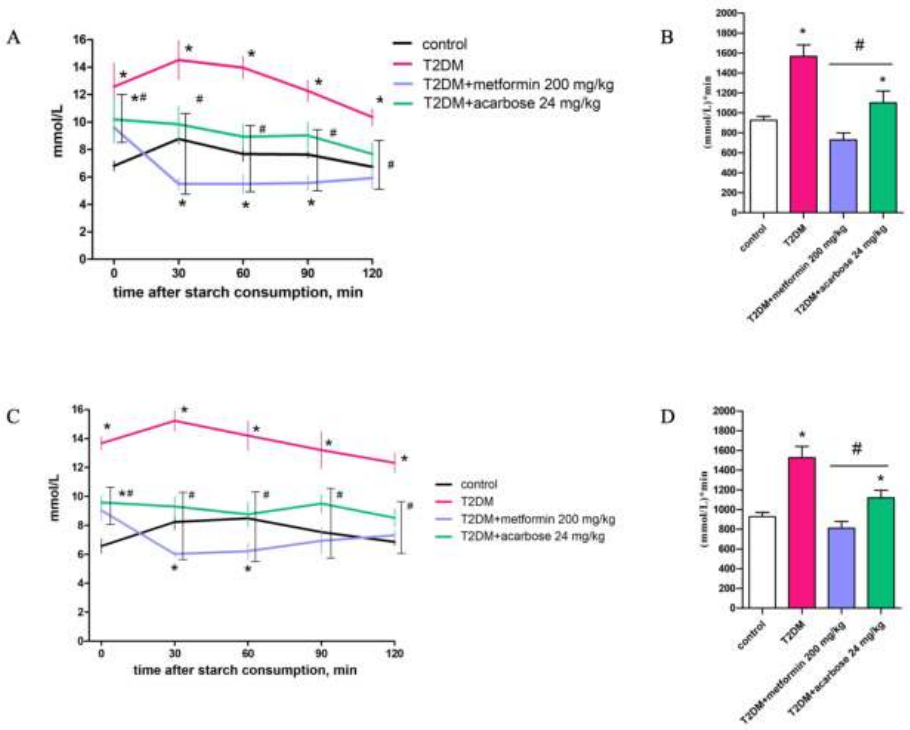


Figure 1. Glucose tolerance starch test. (**A**) Absolute values of glucose concentration and (**B**) glucose AUC at study week 19 – 2 weeks of test substances administration, and (**C**) absolute values of glucose concentration and (**D**) glucose AUC at study week 21 – 4 weeks of test substances administration.

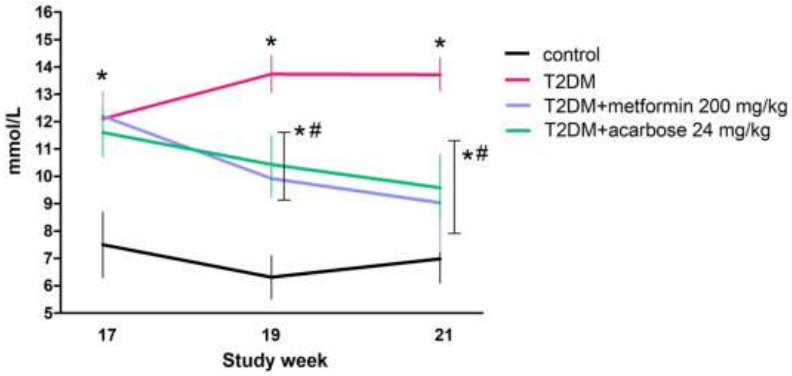


Figure 2. Fasting blood glucose concentration after 2 and 4 weeks of test substances administration.

However, the antihyperglycemic efficacy of acarbose at a dose of 24 mg/kg was not superior to that of metformin at a dose of 200 mg/kg.

Fasting blood glucose concentration

The glucose concentration in animals with T2DM at week 19 of HFD averaged 12.1 mmol/L, and at week 21 it averaged 13.7 mmol/L (Fig. 2). Acarbose at a dose of 24 mg/kg effectively reduced fasting glucose levels after 2 and 4 weeks of daily oral administration on par with metformin. After 2 weeks of metformin and acarbose administration, fasting glucose levels were 9.9 mmol/L and 10.4 mmol/L, and after 4 weeks of administration – 9.0 mmol/L and 9.5 mmol/L, respectively.

Insulin resistance test

HFD in C57BL/6 mice for 19 and 21 weeks resulted in a significant glucose concentration increase in the IRT after insulin administration relative to the control group. A pronounced increase in absolute values of glucose concentration relative to the control group was observed 120 minutes after insulin administration. The glucose AUC was also increased by 50% at week 19 of the study and by 42% at week 21 of the study (Fig. 3).

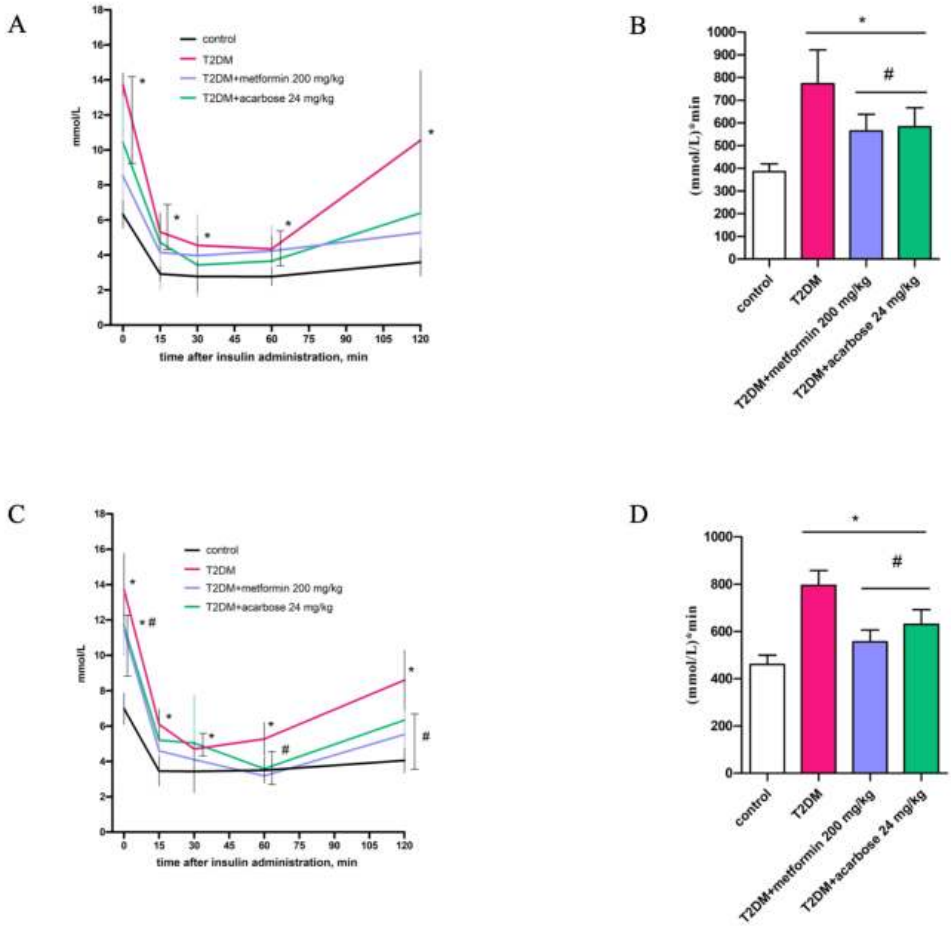


Figure 3. Insulin resistance test. (**A**) Absolute values of blood glucose concentration and (**B**) glucose AUC at study week 19 – 2 weeks of test substance administration, and (**C**) absolute values of blood glucose concentration and (**D**) glucose AUC at study week 21 – 4 weeks of test substances administration.

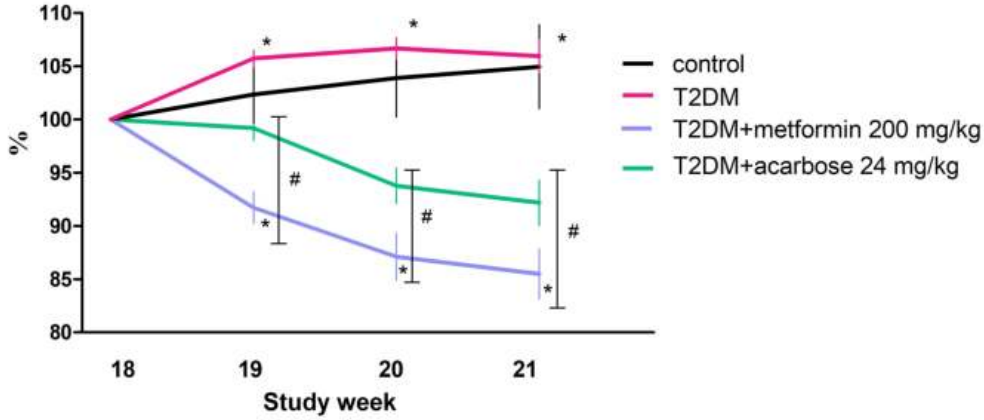


Figure 4. Body weight gain relative to week 18 of the study.

Administration of acarbose at a dose of 24 mg/kg for 2 and 4 weeks resulted in a significant glucose AUC decrease, similar to metformin at a dose of 200 mg/kg. The absolute values of glucose concentration after acarbose administration for 4 weeks were significantly reduced at 60 and 120 minutes in the IRT relative to the T2DM group.

Body weight gain

In the T2DM model, animals continued to gain weight from week 18 of the study, with a 5% weight gain by week 21 of the study. Treatment with acarbose for 4 weeks significantly reduced body weight gain to 92% of 18th week (Fig. 4).

Administration of metformin at a dose of 200 mg/kg was more pronounced than acarbose in reducing weight gain – after 4 weeks of administration, weight gain after metformin administration averaged 85%, and after acarbose administration – 94% of the initial weight values at week 18.

Visceral fat surrounding epididymis

At week 17 of the study, visceral fat mass was significantly increased in animals on HFD relative to body weight, probably due to an increase in visceral fat adipocyte diameter up to 94 μm. At week 21 of the experiment, animals in the model group had an average visceral fat mass ratio of 5.6% and an adipocyte diameter of 107 μm, while the control group had an average visceral fat mass ratio of 1.5% and an adipocyte diameter of 50 μm (Fig. 5).

Administration of acarbose at a dose of 24 mg/kg for 4 weeks resulted in a significant reduction in adipocyte diameter, while visceral fat mass ratio remained at the level of untreated T2DM animals. In contrast, metformin administration significantly reduced both visceral fat mass ratio and adipocyte diameter.

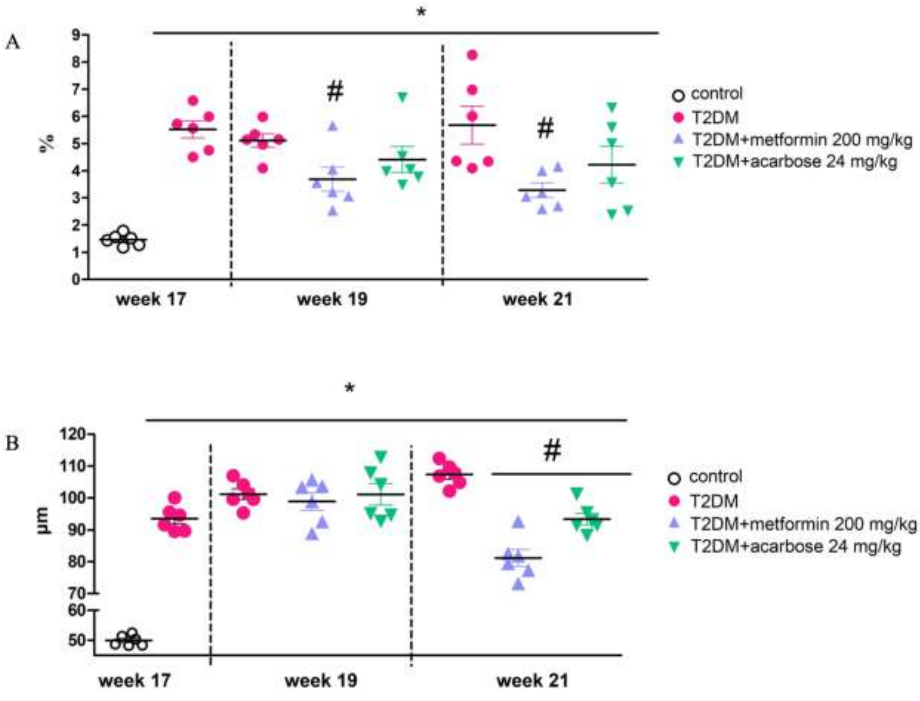


Figure 5. Visceral fat surrounding epididymis. (**A**) Fat mass ratio relative to body weight and (**B**) adipocyte diameter.

Discussion

In T2DM therapy, it is important to control postprandial glucose concentration in order to reduce the formation of glycation end products, which have been identified as a major risk factor for cardiovascular complications in diabetic patients (Tomic et al. 2022). Acarbose slows down the breakdown of starch, and therefore reduces the peak blood glucose concentration after oral starch consumption. However, the efficacy of acarbose in T2DM therapy is not limited to this. New benefits of acarbose have been reported through indirect effects on GLP-1 secretion (Dalsgaard et al. 2021).

It has been hypothesized that acarbose will correct carbohydrate and lipid metabolism – reducing insulin resistance and fasting glucose levels, and preventing the development of obesity. This effect of α-amylase inhibitors suggests their efficacy with repeated chronic intake.

Indeed, our results demonstrate the efficacy of 2- and 4-weeks monotherapy with acarbose at 24 mg/kg in C57BL/6 mice with experimental T2DM regarding glucose metabolism. We observed not only a reduction in postprandial glucose levels in the starch glucose tolerance test, but also in fasting glucose levels and a reduction in insulin resistance. The Acarbose Cardiovascular Evaluation (ACE) study also showed a reduction in fasting glucose levels in people with T2DM taking acarbose (Gerstein et al. 2020).

Similar to our experimental findings of preventing obesity in mice with experimental T2DM, a clinical trial showed the efficacy of acarbose in improving waist-to-height ratio (Song et al. 2020).

Conclusion

Thus, acarbose at a daily dose of 24 mg/kg contributes to the reduction of insulin resistance, fasting glucose concentration and obesity in experimental T2DM, which is probably associated with indirect stimulation of GLP-1 secretion. We suggest the possibility of using α-amylase inhibitors as second-line therapy, as monotherapy at the start of T2DM treatment or as preventive therapy in pre-diabetes. α-Amylase inhibitors are the least studied second-line drugs in T2DM therapy. The effect of α-amylase inhibitors on the progression of T2DM and the development of microvascular and macrovascular complications is still an open problem.

Conflict of interest

The authors have declared that no competing interests exist.

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Data availability

All of the data that support the findings of this study are available in the main text.

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