Long-Term Administration of Dehydroepiandrosterone Accelerates Glucose Catabolism via Activation of PI3K/Akt-PFK-2 Signaling Pathway in Rats Fed a High-Fat Diet

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Abstract

Dehydroepiandrosterone (DHEA) has a fat-reducing effect, while little information is available on whether DHEA regulates glucose metabolism, which would in turn affect fat deposition. To investigate the effects of DHEA on glucose metabolism, rats were administered a high-fat diet containing either 0 (HCG), 25 (HLG), 50 (HMG), or 100 (HHG) mg·kg\(^{-1}\)·day\(^{-1}\) DHEA per day via gavage for 8 weeks. Results showed that long-term administration of DHEA inhibited body weight gain in rats on a high-fat diet. No statistical differences in serum glucose levels were observed, whereas hepatic glycogen content in HMG and HHG groups and muscle glycogen content in HLG and HMG groups were higher than those in HCG group. Glucokinase, malate dehydrogenase and phosphofructokinase-2 activities in HMG and HHG groups, pyruvate kinase and succinate dehydrogenase activities in HMG group, and pyruvate dehydrogenase activity in all DHEA treatment groups were increased compared with those in HCG group. Phosphoenolpyruvate carboxykinase and glycogen phosphorylase mRNA levels were decreased in HMG and HHG groups, whereas glycogen synthase-2 mRNA level was increased in HMG group compared with those in HCG group. The abundance of Glut2 mRNA in HMG and HHG groups and Glut4 mRNA in HMG group was higher than that in HCG group. DHEA treatment increased serum leptin content in HMG and HHG groups compared with that in HCG group. Serum insulin content and insulin receptor mRNA level in HMG group and insulin receptor substrate-2 mRNA level in HMG and HHG groups were increased compared with those in HCG group. Furthermore, Pi3k mRNA level in HMG and Akt mRNA level in HMG and HHG groups were significantly increased than those in HCG group. These data showed that DHEA treatment could enhance glycogen storage and accelerate glucose catabolism in rats fed a high-fat diet, and this effect may be associated with the activation of PI3K/Akt-PFK-2 signaling pathway.
Introduction

Obesity poses a very serious threat to human health [1, 2], and it is associated with a number of metabolic diseases, such as chronic diabetic hyperglycemia, diabetes mellitus, hypertension, and fatty liver disease [3–6]. These diseases affect millions of individuals who must carefully control their blood glucose levels to prevent diabetes-related complications [6]. Increased intake of calorie-rich foods and a sedentary lifestyle are the main causes of obesity in humans worldwide [7]. In addition to exercise, healthy foods and food ingredients may be a practical way to control body weight and fat accumulation [8]. Although numerous studies have focused on various approaches to reduce body weight and control fat accumulation via administration of bioactive compounds [9–12], recently, there has been increasing concern on obesity associated with glycometabolism [4, 5, 13, 14].

Dehydroepiandrosterone (DHEA), a naturally occurring steroid, is mainly secreted by the adrenal cortex [15]. One characteristic of DHEA production is its age-associated production [16]; its aged-related decline has attracted attention with regard to physical health [17, 18]. Currently, DHEA is commercially available as a non-prescription nutritional supplement [19]. The administration of DHEA reduces body weight gain and visceral fat accumulation [11, 20]. Our laboratory previously showed that DHEA accelerated lipid catabolism by the activating of the cAMP-PKA signaling system [21], which will induced the expression of relevant genes expression [22]. It is well known that there is a close relationship between glucose metabolism and fat metabolism in the body. Administration of DHEA, a potential therapy for weight loss and fat accumulation reduction, may be a practical way to reduce body weight and excessive fat in humans or animals. However, little information is available to assess whether DHEA regulates glucose metabolism, which would in turn affect body weight and fat deposition.

Therefore, the present study was conducted to investigate the effects of the long-term administration of DHEA on glucose metabolism and its consequence in rats fed a high-fat diet. This information will deepen our understanding of the mechanisms driving DHEA and verify it as a nutritional supplement to control body weight and to curb obesity-related diseases.

Materials and Methods

Animals and dietary treatment

Two-month-old Sprague Dawley rats weighing 200 ± 20 g were purchased from the Experimental Animal Center of the Jiangsu University (China). All animal handling procedures were performed in strict accordance with the Care and Use of Laboratory Animals central of the Nanjing Agricultural University guidelines. The protocol was approved by the Institutional Animal Care and Use Committee of the Nanjing Agricultural University. Rats were housed individually under a constant temperature of 25°C and humidity ranged between 50–60%, with a 12 h light: 12 h dark cycle. Animals were treated as indicated in Fig 1. Briefly, after one week of aclimatization, 75 rats were randomized into five groups: normal diet control group (NCG), high-fat diet control group (HCG), high-fat diet with low dose DHEA group (HLG), high-fat diet with medium dose DHEA group (HMG), and high-fat diet with high dose DHEA group (HHG). Rats in the NCG group were fed a normal diet, whereas those in the other groups were fed a high-fat diet with the corresponding DHEA treatment (purchased from Changzhou Jiaerke Pharmaceuticals Group Corp., and dissolved in 1% DMSO) via gavage at 0, 25, 50, and 100 mg·kg⁻¹ body weight (for HCG, HLG, HMG, and HHG, respectively) once per day for 8 weeks. The NCG group rats received an equal volume of placebo each day for eight weeks. The normal diet (3.57 kcal·g⁻¹ formula comprising, 24.6% protein, 16.4% fat, and 59% carbohydrates; GB14924, 3–2010, China) and high-fat diet (4.45 kcal·g⁻¹ formula comprising, 18% protein, 44% fat, and 38% carbohydrates) were provided by the Jiangsu Xietong Biotechnology
Institution (China). At the end of the experiment, food was removed for the final 12 hours, and then the rats were anesthetized with ether and sacrificed by decapitation. Blood samples were allowed to clot at 4°C and were then centrifuged at 1520 × g for 20 min before harvesting the serum. The serum, liver and muscle samples were collected and kept at -70°C until further analysis.

Measurement of serum glucose and glycogen content

Serum glucose, hepatic glycogen, and muscle glycogen contents were measured using commercial kits according to the manufacturers’ protocols (Nanjing Jiancheng Biotechnology Institution, China).

Measurement of key enzyme activity during glycometabolism in the liver

The activities of glucokinase (GK), pyruvate kinase (PK), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH) in the liver were measured using commercial kits according to the manufacturers’ protocols (Nanjing Jiancheng Biotechnology Institution, China). The activities of pyruvate dehydrogenase (PDH, E1) and phosphofructokinase-2 (PFK-2) in the liver were measured using ELISA kits according to the manufacturers’ protocols (Shanghai Lengton Bioscience Co., China).

Measurement of serum hormone content

Serum insulin and leptin contents were measured using radioimmunoassay kits according to the manufacturers’ protocols (Beijing North Institute of Biotechnology, China). The intra-coefficients of variation for all hormone detection kits were less than 10%, and inter-coefficients of variation were less than 15%.

Determination of glucose metabolism-related factor gene mRNA levels by real-time PCR

Total RNA was extracted from liver and muscle samples using the TRIZOL reagent (Takara, Japan) according to the manufacturer’s protocol. Total RNA (1 μg) was reverse transcribed.
into cDNA using the Superscript II kit (Invitrogen, USA), according to the manufacturer’s recommendations. An aliquot of cDNA sample was mixed with 25 μL SYBR Green PCR Master Mix (TaKaRa, Japan) in the presence of 10 pmol each of forward and reverse primers for β-actin (used as an internal control), phosphoenolpyruvate carboxykinase (Pepck), glycogen phosphorylase (Pgly), glycogen synthase-2 (Gys2), insulin receptor (Insr), insulin receptor substrate-2 (Irs2), glucose transporter-2 (Glut2), phosphatidylinositol 3-kinase (Pi3k), and protein kinase B (Akt) in liver and glucose transporter-4 (Glut4) in muscle tissues. All samples were analyzed in duplicate in an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Stockholm, Sweden) and programmed to conduct one cycle (at 95°C for 1 min) and 40 cycles (of 95°C for 30 s, 60°C for 30 s, and 72°C for 40 s). Fold change was calculated using the $2^{-\Delta\Delta CT}$ method, and the relative amount of mRNA for each target gene was determined by calculating the ratio between each mRNA and the mRNA of β-actin [23, 24]. In our experimental system, DHEA treatment did not change the expression of β-actin. The primers used (Table 1) were designed using Primes Premier 5 and synthesized by Invitrogen Biological Company (China).

### Statistical analysis

All statistical analyses were performed with SPSS 17.0 for Windows (StatSoft, Inc., Tulsa, OK, USA), and results are expressed as means ± SE. A t-test was used to compare diet control groups, and a one-way analysis of variance (ANOVA) was used to compare the effects of DHEA concentrations within groups fed the same diet. Differences were considered significant at $P < 0.05$.

### Results

**Impact of DHEA on body weight, body mass index (BMI) and Lee’s index in rats fed a high-fat diet**

Body weight and average daily gain in the HCG group were significantly higher ($P < 0.05$) than in the NCG group (Fig 2). Consumption of the high-fat diet resulted in a greater degree of

### Table 1. Primer sequences for β-actin and other target genes.

| Gene    | GenBank accessionNumber | Primer sequences (5'–3') | Orientation |
|---------|-------------------------|--------------------------|-------------|
| β-actin | NM_031144               | GATTACTGGCTGGCTCTCTTA    | Forward     |
|         |                         | TCATCTGACTGCCAGCTTCTCA   | Reverse     |
| Pi3k    | NM_053481               | CGGGGACACCTGTTACACG      | Forward     |
|         |                         | ACGGGTGGTGGATCACAGG      | Reverse     |
| Akt     | NM_033230               | TCAGAACCATGGCCAGTATC     | Forward     |
|         |                         | TGCTACATCAAAGCCAGTC     | Reverse     |
| Pepck   | NM_198780               | GCTGCGAAGAGACGAGAAAG    | Forward     |
|         |                         | AATCCGGGCGGAGGAAAG      | Reverse     |
| Gys2    | NM_013089               | TGCCCTTGGACAGAGTGGTAC   | Forward     |
|         |                         | TGGATGTGGTGGTCAGGAG      | Reverse     |
| Inrs    | NM_017071               | GCAGAGAGACTCATGCTGCT    | Forward     |
|         |                         | CCATACCCGCGCCATGCTGCC   | Reverse     |
| Glut2   | NM_012879               | TGCTGGAAGAAGACGCTACAG   | Forward     |
|         |                         | GGCCAAAGTGAGATGCTGAG     | Reverse     |
| Glut4   | NM_012751               | TGGTGCCGATGCTATG        | Forward     |
|         |                         | CTGCGAGGAAAGGAGGGA      | Reverse     |
| Irs2    | AF087674                | CATCGTGAGAAGCAGTACAG    | Forward     |
|         |                         | GACCAGGCGGCTGACCGG      | Reverse     |

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Fig 2. Effect of DHEA on body weight, Feed intake, BMI, and Lee’s index in rats fed a high-fat diet. A: Body weight; B: Average daily gain; C: Feed intake; D: Body mass index (BMI); E: Lee’s index. Data are expressed as means ± SE (n = 15). * P < 0.05, compared with the HCG group.

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obesity among rats in the HCG group than in the NCG group ($P < 0.05$), as indicated by significantly higher BMI and Lee’s indices. No statistical differences were observed on feed intake in DHEA treatment groups compared with the HCG group ($P > 0.05$) (Fig 2C). Body weight and average daily gain were markedly reduced in the HMG group compared with the HCG group ($P < 0.05$) (Fig 2A and 2B). In addition, BMI and Lee’s indices were significantly lower in the HMG group than they were in the HCG group ($P < 0.05$) (Fig 2D and 2E).

Effect of DHEA on serum glucose level and metabolism hormone concentration in rats fed a high-fat diet

No statistical differences were observed regarding the effect of DHEA on serum glucose and leptin contents between the HCG and NCG groups ($P > 0.05$), whereas serum glucagon and insulin contents were significantly higher in the HCG group than in the NCG group ($P < 0.05$) (Fig 3). Compared with the HCG group, DHEA treatment tended to decrease serum glucose level in rats fed a high-fat diet ($P > 0.05$) (Fig 3A). DHEA treatment significantly increased

![Graphs showing effect of DHEA on serum glucose, leptin, glucagon, and insulin levels.](https://example.com/graphs.png)

**Fig 3.** Effect of DHEA on glucose content and metabolic hormone in rats fed a high-fat diet. A: Glucose content; B: Leptin content; C: Glucagon content; D: Insulin content. Data are expressed as means ± SE (n = 15). ** $P < 0.01$ and * $P < 0.05$, compared with the HCG group.

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serum leptin content in the HMG and HHG groups compared with those in the HCG group ($P < 0.01$) (Fig 3B). No statistical differences were detected in glucagon content between DHEA treatment groups and HCG group ($P > 0.05$) (Fig 3C). Compared with the HCG group, serum insulin content in the HMG group was significantly higher ($P < 0.05$) (Fig 3D).

**Effect of DHEA on glycogen content and glycogen metabolism in rats fed a high-fat diet**

Hepatic and muscle glycogen contents in the HCG group were significantly higher compared with the NCG group ($P < 0.01$) (Fig 4A and 4B). The hepatic glycogen contents were significantly higher in the HMG and HHG groups than that in the HCG group ($P < 0.01$) (Fig 4A). Compared with the HCG group, muscle glycogen contents in the HLG and HMG groups were significantly higher in the high-fat diet-fed rats ($P < 0.01$) (Fig 4B). In addition, $Pygl$ mRNA levels were significantly higher in the HCG group than that in the NCG group ($P < 0.01$) (Fig

![Graph A: Hepatic glycogen content (mg/g)](https://via.placeholder.com/150)

![Graph B: Muscle glycogen content (mg/g)](https://via.placeholder.com/150)

![Graph C: $Pygl$ mRNA expression level](https://via.placeholder.com/150)

![Graph D: Gys2 mRNA expression level](https://via.placeholder.com/150)

**Fig 4. Effect of DHEA on glycogen contents and mRNA expression of key glycogen metabolism enzymes in rats fed a high-fat diet. A: Hepatic content; B: Muscle content; C: Glycogen phosphorylase ($Pygl$) mRNA level in liver; D: Glycogen synthase-2 ($Gys2$) mRNA level in liver. Data are expressed as means ± SE (n = 15). ** $P < 0.01$, compared with the HCG group.

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4C), whereas no significant difference was observed on Gys2 mRNA level in the HCG group when compared to the NCG group ($P > 0.05$) (Fig 4D). Compared with the HCG group, administration of DHEA significantly decreased Pygl mRNA levels in rats fed a high-fat diet ($P < 0.01$) (Fig 4C). However, Gys2 mRNA levels were significantly higher in the HMG group than that in the HCG group ($P < 0.05$) (Fig 4D).

Effect of DHEA on glucose catabolism in rats fed a high-fat diet

No significant differences were observed in the GK, PDH (E1), SDH, and PFK-2 activities between the HCG and NCG groups ($P > 0.05$), while MDH and PK activities in the HCG group were significantly higher than those in the NCG group ($P < 0.05$) (Fig 5). The GK activity in the HMG and HHG groups ($P < 0.05$) (Fig 5A) and PK activity in the HMG group ($P < 0.05$) (Fig 5B) were significantly increased compared with those in the HCG group. Compared with the HCG group, administration of DHEA significantly increased PDH (E1) activity in rats fed high-fat diet ($P < 0.05$) (Fig 5C). Similarly, SDH activity in the HMG group ($P < 0.05$) (Fig 5D) and MDH activity in the HMG and HHG groups ($P < 0.01$) (Fig 5E) were significantly increased when compared with the HCG group. In addition, we found that PFK-2 activities were significantly increased in the HMG and HHG groups compared with that in the HCG group ($P < 0.05$) (Fig 5F).

Effect of DHEA on gluconeogenesis in rats fed a high-fat diet

No significant differences were observed in Pepck mRNA levels between the HCG and NCG groups ($P > 0.05$) (Fig 6). However, the Pepck mRNA levels were significantly decreased in the HMG ($P < 0.05$) and HHG ($P < 0.01$) groups compared with the HCG group (Fig 6).

Effect of DHEA on glucose transporters in rats fed a high-fat diet

As shown in Fig 7, Glut4 mRNA levels in the muscle were significantly higher in the HCG group than that in the NCG group ($P < 0.01$), whereas no differences were observed in Glut2 mRNA level in the livers between the HCG and NCG groups ($P > 0.05$). DHEA treatment significantly increased Glut2 mRNA level in the livers in the HMG and HHG groups compared with the HCG group ($P < 0.01$) (Fig 7A). In addition, the Glut4 mRNA level in the HMG group was significantly higher in the muscle than that in the HCG group ($P < 0.01$) (Fig 7B).

Effect of DHEA on insulin receptor and insulin receptor substrate mRNA levels in rats fed a high-fat diet

No significant differences were observed on Insr and Irs2 mRNA levels in livers between the HCG and NCG groups ($P > 0.05$) (Fig 8). Inrs mRNA level was significantly higher in the HMG group than that in the NCG group ($P < 0.05$) (Fig 8A). In addition, no significant differences were observed in Irs1 mRNA levels, whereas Irs2 mRNA levels in the HMG and HHG groups were significantly higher than that in the HCG group ($P < 0.05$) (Fig 8B and 8C).

Effect of DHEA on Pi3k and Akt mRNA levels in the liver of rats fed a high-fat diet

As shown in Fig 9, no changes were observed in Pi3k and Akt mRNA levels between the HCG and NCG groups ($P > 0.05$). DHEA treatment significantly increased Pi3k mRNA levels in the HMG group compared with the HCG group ($P < 0.05$) (Fig 9A). The Akt mRNA levels in the HMG and HHG groups were significantly higher than that in the HCG group ($P < 0.05$) (Fig 9B).
Fig 5. Effect of DHEA on key enzymes activities in the liver of rats fed a high-fat diet. A: Glucokinase (GK) activity; B: Pyruvate kinase (PK) activity; C: Pyruvate dehydrogenase (PDH) activity; D: Succinate dehydrogenase (SDH) activity; E: Malate dehydrogenase (MDH) activity; F: Phosphofructokinase-2 (PFK-2). Data are expressed as means ± SE (n = 15). ** P < 0.01 and * P < 0.05, compared with the HCG group.

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Discussion

Adiposity was determined by Lee’s index, which is defined as the cubic root of body weight in grams divided by the naso-anal length in millimeters multiplied by $10^4$ [10]. It has been shown that BMI and Lee’s index are highly correlated with obesity [10]. Our results showed that Lee’s index was significantly higher in rats under a high-fat diet than in rats on a normal diet. In addition, serum glucose content in rats on a high-fat diet increased by 12.15% relative to that in rats on a normal diet. Therefore, the high-fat diet in our experiment induced obesity in rats, which provides a model for further investigation of the preventative effect of DHEA on obesity in rats and its possible mechanisms.

Our results showed that long-term DHEA administration decreased body weight in rats on a high-fat diet. These results was consistent with a previous report showing long-term DHEA treatment results in the suppression of body weight gain in rodents [25], as well as with the results of Gansler et al. which demonstrated that long-term DHEA administration to lean or...
obese Zucker rats resulted in decreased body weight [26]. Many studies have found that DHEA administration reduces fat accumulation in chickens [22, 27, 28], rodents [29–32] and humans [18, 33]. DHEA has a fat-reducing effect, which may be accomplished through multiple mechanisms [34]. It is well known that fat accumulation relies on excess energy supply in the body. It was reported that DHEA treatment significantly promoted glucose conversion to glycogen, which could be one of the mechanisms controlling serum glucose levels [35]. Our results indicated that no differences were observed in glucose levels, whereas hepatic glycogen contents were markedly higher in the 50 and 100 mg·kg⁻¹ DHEA treatment groups, and muscle glycogen contents were dramatically higher in the 25 and 50 mg·kg⁻¹ DHEA treatment groups. Glycogen is synthesized in response to an increase in blood glucose concentration and processed into glucose to maintain blood glucose homeostasis. Glycogenesis and glycogenolysis during the diurnal cycle are mediated by glycogen synthase and glycogen phosphorylase (PYGL) [36]. Animal body usually express two isoforms of glycogen synthase, of which glycogen synthase-2 (GYS-2) appears to be the most important determinant of glycogen accumulation [37].

**Fig 8.** Effect of DHEA on insulin receptor and insulin receptor substrate mRNA expression in rats fed a high-fat diet. A: Insulin receptor (Irns) mRNA level in liver; B: Insulin receptor substrate-1 (Irs1) mRNA level in muscle; C: Insulin receptor substrate-2 (Irs2) mRNA level in liver. Data are expressed as means ± SE (n = 15). **P < 0.01 and * P < 0.05, compared with the HCG group.

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Administration of different doses of DHEA significantly decreased Pygl mRNA levels, and the 50 mg·kg⁻¹ DHEA treatment significantly increased Gys2 mRNA levels in rats on a high-fat diet. These results indicated that DHEA treatment increased glycogen content by enhancing the expression of glycogen synthase-2 and inhibiting the expression of glycogen phosphorylase.

Gluconeogenesis is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as pyruvate, lactate, glycerol and glucogenic amino acids [38]. Phosphoenolpyruvate carboxykinase (PEPCK) is the rate-limiting enzyme in gluconeogenesis and which catalyzes the glucose synthesis from metabolic precursors [39]. We found that Pepck mRNA expression levels significantly decreased in the 50 and 100 mg·kg⁻¹ DHEA treatment groups. As PEPCK is one of the key enzymes of gluconeogenesis, we speculated that DHEA treatment inhibited the generation of glucose by reducing Pepck mRNA expression.

Several studies have demonstrated that DHEA can promote the absorption of glucose in fibroblasts [40], adipocytes [41], muscle [42] and hepatocytes [43]. In some glucose-sensitive tissues, such as liver and muscle, the transfer of glucose depends on the transporter of GLUT-2 and GLUT-4 [44, 45]. The present study showed that Glut2 mRNA expression levels in the 50 and 100 mg·kg⁻¹ DHEA treatment groups, and Glut4 mRNA expression level in the 50 mg·kg⁻¹ DHEA treatment group significantly increased in the rats on a high-fat diet. This is consistent with a previous report by Sato et al., who found that DHEA activated GLUT-4 protein expression in skeletal muscles [42]. These results suggested that DHEA treatment could promote the absorption of serum glucose by increasing Glut2 and Glut4 mRNA expression levels.

Previous studies have shown that DHEA treatment enhanced the activities of the hexokinase and phosphofructokinase in the glycolytic pathway in the skeletal muscle cells of rats [42]. Thus, we postulate that DHEA might maintain the normal glucose levels by accelerating the glucose catabolism in rats on a high-fat diet. Our results showed that GK activities in the 50 and 100 mg·kg⁻¹ DHEA treatment groups and PK activity in 100 mg·kg⁻¹ DHEA treatment group were significantly enhanced in the liver of rats on a high-fat diet. The pyruvate dehydrogenase complex converts pyruvate into acetyl-CoA, which may then be used in the citric acid cycle for cellular respiration [46]. The reaction catalyzed by pyruvate dehydrogenase (E1) is
considered to be the rate-limiting reaction in the pyruvate dehydrogenase complex [46, 47].
Our results also demonstrated that DHEA treatment significantly increased PDH (E1) activity in the liver of rats on a high-fat diet. SDH is a membrane-bound enzyme, which is the only enzyme that participates in both the citric acid cycle and the electron transport chain [48]. It has been shown that DHEA inhibits NAD-dependent mitochondrial respiration as well as Complex I in the mitochondrial respiratory chain [49], the consequences of which are ATP depletion, increased glucose uptake, and oxidization in the cells to compensate [50]. The present study demonstrated that DHEA-treatment (50 mg·kg\(^{-1}\)) significantly increased SDH activity in the liver of rats on a high-fat diet. These results were similar to our previous study, which established that SDH activity increased in TM-3 cells after DHEA treatment [51]. In addition, MDH activity in the liver significantly increased in the 50 and 100 mg·kg\(^{-1}\) DHEA treatment groups. MDH is an enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate; this reaction is a part of many metabolic pathways, including the citric acid cycle [52]. Taken together, the results indicate that DHEA may maintain normal glucose levels by enhancing some key enzyme activity to accelerate glucose catabolism in rats on a high-fat diet.

Hormones, especially insulin and glucagon, play important roles in controlling blood glucose levels in mammals. No changes were observed in glucagon content, whereas the 50
mg kg\(^{-1}\) DHEA treatment significantly increased insulin content and insulin receptor (Inrs) mRNA expression levels in rats on a high-fat diet. Numerous studies have shown that DHEA treatment significantly increases serum insulin and leptin contents [23, 32]. Insulin is known to upregulate Akt phosphorylation via PI3K, which results in an increase in glucose uptake and utilization [24, 53]. Sato et al. reported that glucose metabolism-related signaling pathways and enzyme activity were enhanced after DHEA supplementation, and that they increased Akt and protein kinase C phosphorylation levels in skeletal muscles [42]. In addition, the 50 and 100 mg kg\(^{-1}\) DHEA treatment significantly increased serum leptin content. Leptin and insulin play similar roles in the PI3K/Akt signaling pathway because the receptor substrate in both processes is insulin receptor substrate-2 (IRS-2) [54, 55]. When insulin binds to the insulin receptor (INSR), IRS-1 in muscle tissue and IRS-2 in liver tissue are activated, which subsequently activate the PI3K/Akt signaling pathway [56, 57]. Our findings indicated that the Inrs, Irs2, Pi3k, and Akt mRNA levels were significantly enhanced by DHEA administration in rats on a high-fat diet. These results indicated that DHEA treatment accelerate glucose catabolism may be associated with the activation of PI3K/Akt signaling pathway in rats fed high-fat diet. Akt promotes glucose transport and stimulates glycolysis through the activation of several glycolytic enzymes, including hexokinase and phosphofructokinase (PFK) [54, 58]. PFK-2 is the sole enzyme responsible for the production and degradation of fructose 2,6-bisphosphate, which allosterically activates PFK-1 more potently than its own product, fructose 1,6-bisphosphate [59]. In addition, PFK-2 may bind and directly activate GK to control the flux of fructose 6-phosphate substrate into fructose 1,6-bisphosphate [60, 61]. In this study, the 50 and 100 mg kg\(^{-1}\) DHEA treatment significantly enhanced PFK-2 activity. As PFK-2 is one of the key enzymes of glycolysis, we speculated that treatment with DHEA activated the PI3K/Akt signaling pathway, which accelerated glucose catabolism by promoting PFK-2 activity.

In conclusion (as shown in Fig 10), our findings demonstrated that DHEA treatment could promote glycogen storage and accelerate glucose catabolism in rats on a high-fat diet. Notably, DHEA treatment accelerated glucose catabolism by promoting PFK-2 activity in rats on a high-fat diet, which may be associated with the activation of PI3K/Akt signaling pathway. Certainly, further investigation should be focused on detecting the protein and phosphorylation levels, or blocking PI3K/Akt pathway to support this conclusion.

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Author Contributions
Conceived and designed the experiments: HTM. Performed the experiments: JK. Analyzed the data: CYG LY. Contributed reagents/materials/analysis tools: LLL. Wrote the paper: JK CYG.

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