Journal of Clinical and Medical Images

Research Article

ISSN: 2640-9615 | Volume 7

Effects of Hyper Methionine (HM) Feeding on Biochemical Indicators in Streptozotocin (STZ) Induced Diabetes Rats

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Received: 26 Feb 2024
Accepted: 06 Apr 2024
Published: 12 Apr 2024
J Short Name: JCMI

Citation:

Cao Y, Ma L, Li P, Effects of Hyper Methionine (HM) Feeding on Biochemical Indicators in Streptozotocin (STZ) Induced Diabetes Rats. J Clin Med Img. 2024; V7(13): 1-6

Keywords:

High methionine (HM) diet; hyperhomocysteinemia (HHcy); diabetes mellitus; liver function; kidney function.

1. Abstract

Elevated Hcy levels are associated with diabetes mellitus and its complications, though a high methionine (HM) diet can lead to hyperhomocysteinemia (HHcy), few have investigated the impact of the HM diet other than HHcy. In this paper, we fed 2% methionine to either normal 8-week-old Wistar rats or streptozotocin-induced diabetes Wistar rats (HM+STZ group) for 12 weeks to investigate the effects of the HM diet on blood glucose, blood lipid, liver function, and kidney function. HM feeding can significantly increase Hcy levels either in the normal rats or in the STZ rats, though it has no effect on blood glucose. Interestingly, the HM diet could reduce triglyceride (TG) and alanine aminotransferase (ALT) levels in the STZ rats. At 4, 8 and 12 weeks, the 24-hour urinary albumin levels in the HM+STZ group (17.12 ± 8.44 mg/L, 24.37 ± 14.09 mg/L, 26.59 ± 17.63 mg/L) were significantly higher than those of the STZ group (7.76 ± 3.92 mg/L, 7.92 ± 4.23 mg/L, 11.66 ± 1.88 mg/L, $P<0.05$). Compared with the STZ group (9.30 ± 2.92 mg/g, 108.88 ± 58.70 mg/g, 238.41 ± 62.52 mg/g), urinary albumin/creatinine levels in the HM+STZ group (21.37 ± 12.13 mg/g, 127.30 ± 81.33 mg/g, 411.90 ± 88.86 mg/g) significantly increased ($P<0.05$) from the 4_{th} week which are consistent with renal morphological changes. These findings suggest that there is a certain correlation between HM diet and blood lipid, liver function, and kidney function, which may be an important risk factor for aggravating the progress of DN.

2. Introduction

Homocysteine (Hcy) is a kind of sulphur-containing amino acid which is the intermediate product of the methionine metabolism [1]. Demethylation of methionine is an important metabolic activity that affects the body's methylation reactions by providing methyl groups [2]. There are two main pathways for Hcy metabolism: one is the regeneration of methionine under the action of methionine synthase (MS) and methylenetetrahydrofolate reductase (MTHFR) using vitamin B12 as a coenzyme; the second pathway is the synthesis of β- Cystine 2 under the action of cysteine sulfide synthase (CBS) and vitamin B6 as a coenzyme. The production and clearance of Hcy maintain a dynamic balance in the normal body, and the plasma concentration remains at 5-15 μ mol/L approximately. When the concentration of peripheral blood Hcy exceeds 100 μmol/L, it is called hyperhomocysteinemia (Hhcy) [3]. Excessive intake of methionine, auxiliary factors related to homocysteine metabolism or lack of enzymes that lead to homocysteine metabolism disorder will lead to the accumulation

of Hcy in the circulation [4]. Currently, a high methionine diet has been used to induce HHcy in rodents [5].

Most studies believe that the presence of HHcy in the circulation of diabetes patients is a powerful driving factor for the occurrence and development of diabetic chronic complications [6, 7]. Elevated homocysteine (Hcy) concentration may be associated with a decrease in glomerular filtration rate (GFR) in patients with diabetic nephropathy (DN). Since the kidney is the primary organ for clearing and metabolizing Hcy [8], reduced GFR in the kidney may lead to the accumulation of Hcy in the body. Thus, HHcy is considered a key risk factor for the development of DN in diabetic patients. Until now, we have rarely known about the effects of HHcy on blood glucose, liver and kidney functions. In this paper, we investigated the effects of HHcy induced by high methionine (HM) feeding on liver and kidney functions of normal rats and Streptozotocin (STZ) -induced diabetic rats. STZ can destroy pancreatic tissue and result in an absolute lack of insulin secretion, leading to diabetes in rats. 2% methionine feeding was given to the normal male 8-week-old Wistar rats and STZ-induced diabetic Wistar rats for 12 weeks.

3. Materials and Methods

3.1. Animal Modeling

The research has been approved by the Animal Ethics Committee of China-Japan Friendship Hospital. Experimental animals' care and use was in accordance with the ARRIVE guidelines. Male 8-week-old Wistar rats (weight 250–300g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). One week has been given for animals to adapt to the environment. In order to avoid the risk of bias, the experimental animals were randomly grouped. The rats were then divided into four groups: the normal group (n = 8) was fed with the normal diet until the end of the experiment as a control. The hyper-methionine group (HM group, n = 8) was fed a 2% -methionine diet for 12 weeks to investigate the effects of HM feeding on normal rats. The STZ group (n = 8) was induced by intraperitoneal administration of 1% STZ (30 mg/kg, STZ was diluted in 0.1 mol/L citrate buffer at pH 4.5) twice within one-week. A blood glucose level of >16.7 mmol/L in the caudal vein for three consecutive days after the second STZ injection was considered a standard for the diabetic model. The STZ group received normal feed and no other treatment. The HM+STZ group (n = 8) was induced diabetes by the same method of STZ group, however, a 2% hyper-methionine diet was given for 12 weeks to investigate the effects of HM feeding on diabetic rats. Blood glucose, liver function, and kidney function were monitored before treatment and every four weeks after treatment in all groups.

3.2. HCY-related Indicators and Blood Lipid

The levels of Homocysteine (Hcy) and its metabolism-related indicators (FA, vitB12), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) in rats' plasma were measured using the Roche biochemical instrument by the manufacturer's instructions.

3.3. Blood Glucose and Liver Function Determination

The levels of fasting blood glucose (FBG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), and A/G (ALB/GLB) in rat plasma were measured using the Roche biochemical instrument by the manufacturer's instructions.

3.4. Renal Function Detection

At weeks 0, 4, 8, and 12, the rats in each group were placed in a metabolic cage to collect their urine for 24 hours. The levels of 24-hour urine albumin and urine creatinine were measured using the Roche biochemical instrument by the manufacturer's instructions. And the 24-hour urine albumin/creatinine ratio was calculated.

3.5. Histopathological Stains

To observe morphology changes in renal tissue, rats' kidneys of each group were harvested for histopathological staining after 12 weeks treatment. Kidney tissues were fixed in 10% formalin for 48 hours and then paraffin embedded. Each paraffin- embedded sample was cut into 3 μ m thick sections and stained with hematoxylin and eosin (H&E) as well as Schiff (PAS) and Masson reagent.

3.6. Statistical Analysis

The comparisons of various liver and kidney indicators among multiple groups were compared by One-way ANOVA using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). Results were expressed as mean ± standard deviation (x ± s). A p-value less than 0.05 was considered statistically significant.

4. Results

4.1. The HM Diet-induced HHcy in Wistar Rats

To evaluate the effect of HM diet on homocysteine (Hcy) concentration, Wistar rats were fed either normal chow or an HM diet for 12 weeks. Compared with the control group, the rats in the HM group and the HM+STZ group showed a significant increase in Hcy from 4 weeks after HM feeding. The Hcy levels in the HM group were 107.79 ± 50.25 umol/L, 75.11 ± 32.86 umol/L, and 64.08 ± 34.34 umol/L at the 4_{th}, 8_{th}, and 12_{th} weeks, respectively, which were significantly higher than those in the normal chow-fed rats (11.75 ± 2.43 umol/L, 10.13 ± 1.94 umol/L, and 8.53 ± 2.70 umol/L) ($P<0.05$). Since the fourth week of HM feeding, the Hcy levels in the HM+STZ group were 70.40 ± 34.70 umol/L, 104.99 ± 38.95 umol/L, and 72.35 ± 46.98 umol/L, respectively, which were significantly higher than those in the STZ group ($P<0.05$) but not significantly different from those in the HM group ($P>0.05$, see Figure 1A). No significant changes were observed in serum folate

(FB, Figure 1B) and vitamin B12 (vitB12, Figure 1C) related to Hcy metabolism in each group of rats.

We observed interesting data regarding triglyceride (TG) levels. Rats fed a HM diet for 12 weeks showed no significant changes in TG levels when compared to the normal group. However, the levels of TG in the STZ group were significantly higher than those in the normal group at weeks 4, 8, and 12 (4.42 ± 1.25 mmol/L, 4.32 ± 1.23 mmol/L, and 3.97 ± 1.23 mmol/L, respectively, vs. 1.83 ± 0.38 mmol/L, 2.11 ± 1.39125 mmol/L, and 2.14 ± 1.28 mmol/L, respectively) (P<0.05, Figure 1D). Interestingly, the elevated TG levels significantly decreased after being fed with a 2% methionine diet. At the 4th, 8th, and 12th weeks, TG levels in the HM+STZ group were 1.20 ± 0.36 mmol/L, 1.83 ± 0.43 mmol/L, and 1.47 ± 0.43 mmol/L, respectively, and were significantly different from those in the STZ group (P<0.05), but not significantly different from those in the normal group (P>0.05, see Figure 1D). However, the HM diet did not affect the lipid profiles (i.e., TG, HDL-C, and LDL-C) in Wistar rats (Figure 1D-1G).

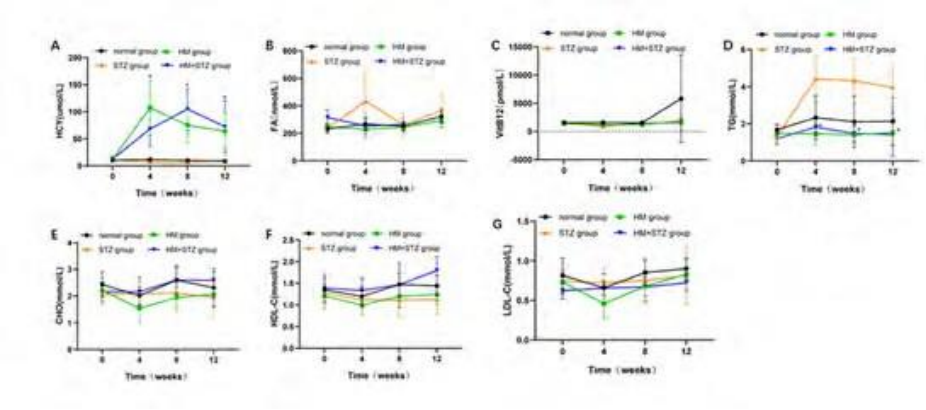

Figure 1: High methionine (HM) diet induces hyperhomocysteinemia (HHcy) in Wistar rats. (A) Plasma Hcy concentration in Wistar rats fed with normal chow or HM diet, and in streptozotocin (STZ)-induced diabetic rats fed with normal chow or HM diet for 12 weeks. (B) Changes in fatty acids (FA) in rats during 12 weeks of feeding. (C) Changes in vitamin B12 levels in rats during 12 weeks of feeding. (D) Changes in triglycerides (TG) in rats during 12 weeks of feeding. (E) Changes in total cholesterol (CHO) in rats during 12 weeks of feeding. (F) Changes in high-density lipoprotein cholesterol (HDL-C) in rats during 12 weeks of feeding. (G) Changes in low-density lipoprotein cholesterol (LDL-C) in rats during 12 weeks of feeding. *P < 0.05 compared to normal chow group. n = 8 per group. All data are expressed as mean ± SD.

4.2. Effect of HM on Blood Glucose and Liver Function

Feeding rats with a 2% methionine diet did not have a significant effect on blood glucose levels. Blood glucose levels in HM group at the 4, 8, and 12 weeks were 7.45 ± 1.27 mmol/L, 9.83 ± 2.55 mmol/L, and 9.90 ± 2.31 mmol/L, respectively, which did not significantly differ from the normal group (P>0.05, refer to Figure 2A). Blood glucose levels in the STZ group rats at weeks 0, 4, 8, and 12 were 24.09 ± 3.88 mmol/L, 30.01 ± 12.73 mmol/L, 25.78 ± 8.14 mmol/L, and 35.18 ± 11.29 mmol/L, respectively, and were significantly higher than those of normal group rats (P<0.05). In the STZ model rats fed a 2% methionine diet, blood glucose levels were 24.84 ± 6.89 mmol/L, 22.72 ± 8.21 mmol/L, 38.79 ± 17.03 mmol/L, and 18.36 ± 6.84 mmol/L at weeks 0, 4, 8, and 12, respectively, which were significantly higher than those in the normal group (P<0.05, refer to Figure 2A) which was no statistically significant difference compared to the STZ group (P>0.05).

Compared to the control group, there were no significant changes in the ALT levels in rats that were fed with the HM diet. However, in the STZ group, the ALT levels were significantly higher than those in the normal group at the 8 and 12 weeks, measuring 277.63 ± 94.68 IU/L and 233.63 ± 106.94 IU/L, respectively (P<0.05, see Figure 2B). For the STZ rats that were fed with 2% methionine, the ALT levels at 8 and 12 weeks were 78.63 ± 9.30 IU/L and 77.5 ± 12.29 IU/L, respectively. There was a significant decrease when compared to the STZ group (P<0.05, see Figure 2B), and there were no significant changes (P>0.05) when compared to the normal group, where the ALT levels were 51.88 ± 13.08 IU/L at week 8 and 47.25 ± 14.42 IU/L at week 12, respectively.

As shown in Figure 2C-2E, there were no statistically significant differences in AST, ALB, TP, and A/G levels among rats in HM group, STZ group, and HM+STZ group compared to the normal group.

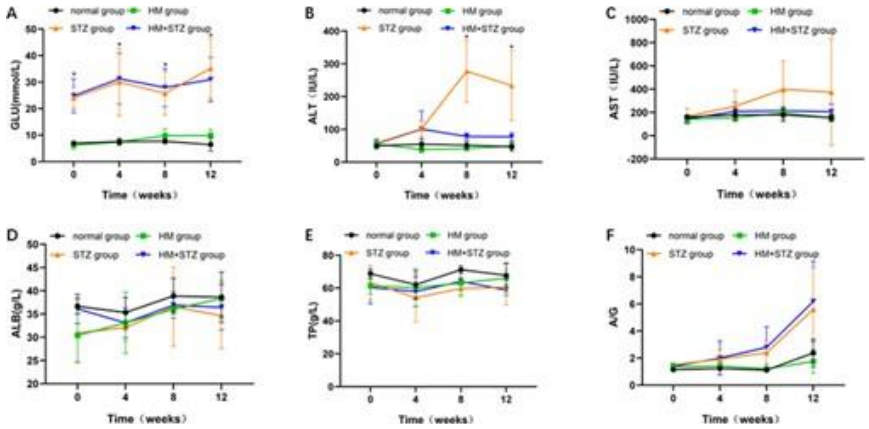

Figure 2: Effects of HM diet on liver function in Wistar rats. (A) Plasma glucose concentrations in Wistar rats fed with either normal chow or HM diet and STZ-induced diabetic rats fed with either normal chow or HM diet for 12 weeks. (B) Changes in ALT levels in Wistar rats over 12 weeks of feeding. (C) Changes in AST levels in rats over 12 weeks of feeding. (D) Changes in ALB levels in rats over 12 weeks of feeding. (E) Changes in TP levels in rats over 12 weeks of feeding. (F) Changes in A/G ratio in rats over 12 weeks of feeding. HM: high methionine; Glu: glucose; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALB: albumin; TP: total protein; A/G: albumin-to-globulin. *P < 0.05 vs normal chow group. n = 8 per group. All data are expressed as mean ± SD.

4.3. Effect of HM on Renal Function

Starting from the 4th week, the 24-hour urinary albumin levels of rats in the HM group were (2.73 ± 1.84 mg/L, 7.29 ± 4.45 mg/L, 9.89 ± 3.67 mg/L); the 24-hour urinary albumin levels of rats in the STZ group were (7.76 ± 3.92 mg/L, 7.92 ± 4.23 mg/L, 11.66 ± 1.88 mg/L); the 24-hour urinary albumin levels of rats in the HM+STZ group were (17.12 ± 8.44 mg/L, 24.37 ± 14.09 mg/L, 26.59 ± 17.63 mg/L) all significantly higher than those of normal group ((0.70 ± 0.67 mg/L, 1.74 ± 1.39 mg/L, 0.92 ± 0.52 mg/L)), with a significant statistical difference (P < 0.05), as shown in Figure 3A. The 24-hour urinary albumin levels in HM group were significantly higher than those of rats in the normal group fed with a normal diet (P < 0.05) and the 24-hour urinary albumin levels in the HM+STZ group were significantly higher than those of rats in the STZ group fed with a normal diet (P < 0.05), suggesting that HHcy may play a role in the changes of renal function in diabetic nephropathy.

During the 12-week period of HM feeding, there was no significant difference in urinary albumin/creatinine levels between the HM group and normal group (P>0.05). In contrast, compared with the normal urinary albumin/creatinine levels in the STZ group and the HM+STZ group significantly increased(P<0.05) from the 4th week, which was 9.30 ± 2.92 mg/g, 108.88 ± 58.70 mg/g, 238.41 ± 62.52 mg/g and 21.37 ± 12.13 mg/g, 127.30 ± 81.33 mg/g, 411.90 ± 88.86 mg/g respectively. We compared urinary albumin/creatinine levels between the STZ group and the HM+STZ group to observe the effect of HM feeding. At week 12, the HM+STZ group had significantly higher urinary albumin/creatinine levels (411.90 ± 88.86 mg/g) than the STZ group (238.41 ± 62.52 mg/g). Figure 3B illustrates the difference in urinary albumin/creatinine levels between the HM+STZ group and the STZ group at week 12. Our findings suggest that, compared with the normal feeding of STZ rats, HM feeding significantly increased urinary albumin/creatinine levels in the STZ rats.

Compared to the normal group, 2% methionine feeding did not have a significant effect on serum urea levels in rats. However, starting from the 4th week, the STZ group (10.18 ± 1.96 mmol/L, 11.72 ± 5.01 mmol/L, 11.88 ± 4.29 mmol/L) and the HM+STZ group (9.24 ± 1.94 mmol/L, 8.69 ± 1.99 mmol/L, 9.95 ± 2.35 mmol/L) both showed a significant increase in urea levels (P<0.05), as presented in Figure 3C. HM feeding and normal feeding had no effect on urea levels in the 24-hour urine of rats with the STZ model of diabetes.

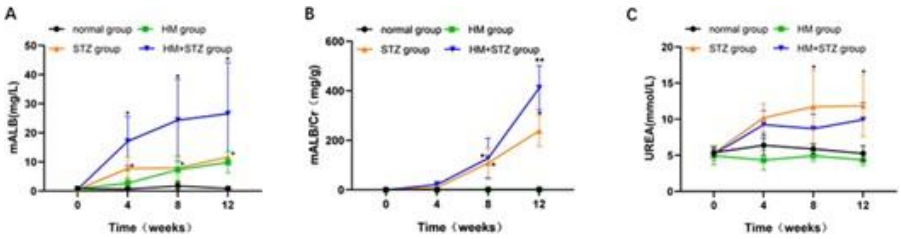

Figure 3: Effects of HM diet on renal function in Wistar rats fed normal chow or HM diet and STZ-induced diabetes rats fed normal chow or HM diet for 12 weeks. (A) mALB of 24h urinary albumin in Wistar rats during 12 weeks of feeding. (B) mALB /Cr of Wistar rats during 12 weeks of feeding. (C) UREA of rats during 12 weeks of feeding. The sample size was n=8 per group, and data are expressed as mean ± SD. *P < 0.05 vs normal chow group.

4.4. Effect of the HM-diet on Glomerular Morphology

The kidneys of STZ group (Figure 1C) and the HM+STZ group (Figure 1D) had more glomerulosclerosis compared to the normal group as seen in the histological examination. The accumulation of extracellular matrix in the mesangium was also observed. HM alone did not have a significant effect on glomerular morphology, which was consistent with the changes in renal function observed in all rat groups (Figure 4).

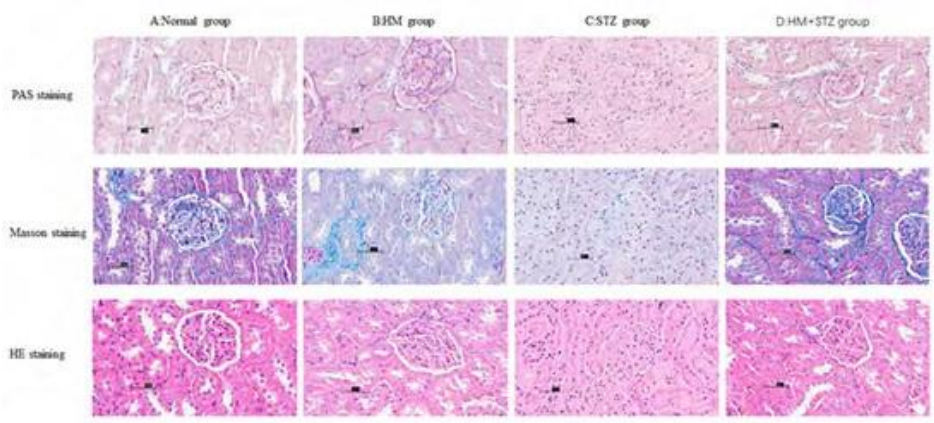

Figure 4: Effects of HM diet on glomerular morphology in Wistar rats (×400). (A)The renal tissue of the normal group was stained with PAS, Masson, and HE after 12 weeks of normal diet feeding. (B)The renal tissue of the HM group was stained with PAS, Masson, and HE after 12 weeks of HM diet feeding. (C)The renal tissue of the STZ group was stained with PAS, Masson, and HE after 12 weeks of normal diet feeding. (D)The renal tissue of the HM+STZ group was stained with PAS, Masson, and HE after 12 weeks of HM diet feeding.

5. Discussion

Hcy is formed during the decomposition of methionine, FA status, vitamin B12, vitamin B6 levels, and renal function are important determinants of plasma Hcy levels. Impaired Hcy metabolism and the occurrence of HHcy may represent a state of increased risk for DN development [9]. HM diets can lead to the accumulation of non-protein amino acid Hcy in the circulation [10].

We set up four groups to explore the effect of high-glucose environments and high methionine feeding on Hcy metabolism: the HM group (normal rats fed a 2% high methionine diet) and the HM+STZ group (rats with STZ-induced diabetes fed a 2% high cholic acid diet) and two control groups (normal group and the STZ group). HM feeding lasted for 12 weeks. Rats in both the HM and the HM+STZ groups showed significantly increased Hcy levels from the 4th week of feeding with 2% methionine, compared to the normal group. However, there was no significant difference in Hcy levels between the HM group and the HM+STZ group (P>0.05, Figure 1A). There was no significant change in Hcy levels between the STZ group and normal group. These findings suggest that the high-glucose model established by STZ had no significant impact on the serum Hcy level. No significant changes were observed in serum folate (Figure 1B) and vitamin B12 (Figure 1C) levels related to Hcy metabolism in any of the rat groups.

We demonstrated that the HM dietary pattern induces HHcy and has multiple effects in vivo. Only the HM diet had no effect on cholesterol levels in rats. In the STZ-induced diabetic rats, serum triglycerides increased significantly from the 4_{th} week of modeling, which is consistent with literature [11]. The HM feeding reduced serum triglyceride content in the STZ-induced diabetic rats, indicating that HHcy represents a different risk factor from high triglycerides.

High methionine supplementation did not affect blood glucose and liver function (ALT, AST, ALB, TP, and A/G) in Wistar rats. STZ induction had no significant impact on AST, ALB, TP, and A/G, but it increased blood glucose [12]. At 8 and 12 weeks of modeling, the ALT of rats in the STZ group was significantly higher than in the normal group, consistent with literature. STZ rats fed with 2% methionine had a significant decrease in ALT at 8 and 12 weeks compared to the STZ group ($P<0.05$, see Figure 2B), and there was no significant difference compared to the normal group ($P>0.05$). 2% methionine feeding may have a protective effect on the liver function of STZ-induced diabetic Wistar rats, but the specific mechanism requires further experiments to elaborate.

The 24-hour urinary albumin and 24-hour urinary albumin/creatinine in th STZ-modeled diabetes rats were significantly higher than those in the normal group from the 4_{th} week, accompanied by changes in glomerular morphology. 2% methionine feeding does not affect the 24-hour urinary albumin and 24-hour urinary albumin/creatinine of normal rats. The 24-hour urinary albumin and 24-hour urinary albumin/creatinine of rats in the HM+STZ group are significantly higher than those in the STZ group, suggesting a correlation between blood Hcy and diabetes nephropathy [13]. High blood Hcy levels may be an important risk factor for aggravating the progress of diabetes nephropathy. 85% to 100% of patients with ESRD have abnormal blood Hcy levels. Hcy causes direct kidney damage through oxidative stress reactions. Hcy promotes the proliferation of vascular smooth muscle cells and the aggregation of platelets in the blood. Hcy similarly affects renal glomerular microvessels, damaging the glomerular molecular filtration barrier and affecting the permeability of the glomerular filtration membrane, leading to the leakage of some large and small molecular proteins [14].

We studied the effects of HM feeding on different rats, including normal rats and STZ diabetes rats. Our focus was on blood glucose, blood lipids, liver function, kidney function, and glomerular morphology. Our findings indicate that HM feeding did not significantly affect blood glucose levels but could reduce TG and ALT levels in the STZ model rats. Additionally, it could worsen diabetes nephropathy in the STZ model rats. We need more research to fully understand the underlying mechanism.

6. Acknowledgments

This study was supported by the National Natural Science Foundation of China (Grant No: 82074221); National High Level Hospital Clinical Research Funding; Elite Medical Professionals project of China-Japan Friendship Hospital (NO.ZRJY2021-GG03); Key Clinical Specialty Project of Beijing (Grant No: 2020).

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