Original Research Article

Effect of sodium butyrate on glucose and lipid metabolism, insulin expression and apoptosis of β-cells in obese pregnant rats

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Abstract

Purpose: To study the influence of sodium butyrate on the metabolism of lipid and glucose, insulin expression and apoptosis of β-cells in obese pregnant rats.
Methods: Three groups of one hundred and twenty 4-week-old female C5BL/6J mice were used: control, high-fat diet and sodium butyrate groups. Insulin, triglycerides and total cholesterol were evaluated by enzyme-linked immunosorbent assay (ELISA). Insulin levels, as well as area and quality of islet β-cells were assessed using Image Pro Plus software. The number of DAPI-positive islet cells, positive expression of bcl-2 in each islet cell, and apoptosis of islet β-cells in each group were determined.
Results: The expression levels of insulin in high-fat diet group and butyrate group were significantly reduced, relative to control, but insulin expression level in Na butyrate group increased, relative to high-fat diet mice ($p < 0.01$). The area and quality of islet β-cells in high-fat diet and sodium butyrate groups were markedly higher in sodium butyrate group than in high-fat diet group ($p < 0.01$). The bcl-2 expression in islet β-cells rose in mice given high-fat diet, relative to control and sodium butyrate groups ($p < 0.01$).
Conclusion: Sodium butyrate facilitates glucose and lipid metabolism, but increases insulin expression, and effectively inhibits apoptosis of islet β-cells in obese pregnant mice. Thus, sodium butyrate may be useful in the prevention and treatment of metabolic disorders due to diabetes mellitus (DM).

Keywords: Sodium butyrate, Obese pregnant mice, Glucose, Lipid metabolism, Islet beta cells, Insulin expression, Apoptosis, Diabetes mellitus

INTRODUCTION

With improvements in standards of living and changes in eating habits in recent years, the incidence of obesity is on the increase in modern industrialized societies [1]. Obesity not only leads to dyslipidemia and metabolic diseases such as type 2 diabetes, hypertension and atherosclerosis, it also increases the risk of cancer, thereby constituting a serious threat to health [2]. Due to the increase in obese, high-society pregnant women, cases of gestational

diabetes (GDM) are also on the increase. Thus, incidents of type 2 diabetes mellitus (T2DM) and obesity in offspring are also significantly increasing [3].

The pathogenesis of GDM is closely related to insufficient level of islet β-cells. It is of great clinical significance to search for drugs for the treatment of metabolic diseases such as diabetes, GDM and T2DM through mechanisms that involve restoration of islet β cells, improvement of the function of islet cells, and inhibition of apoptosis of islet β cells [4]. Sodium butyrate is a naturally occurring histone deacylase inhibitor which enhances the synthesis and release of insulin, and alleviates damage to islet β-cells due to inflammatory response [5]. Sodium butyrate was originally used in studies related to transcriptional regulation of human tumors, but recent studies have found that it is useful in treatment of metabolic diseases, neurodegenerative diseases and inflammatory response-induced diseases [6]. In this study, 120 C5BL/6J female rats (4-week-old) were used to unravel the influence of sodium butyrate on glucose and lipid metabolism, insulin expression and apoptosis of pancreatic beta cells in obese pregnant rats.

EXPERIMENTAL

Animals

A total of 120, 4-week-old female C5BL/6J mice were purchased from the Animal Experimental Center of Shanxi Medical University (license No. SCXK (Jin) 2009-0001). They were housed in a cage at temperature range of 18-22 ℃, and humidity of 55 – 65 %. This animal studies were approved by the Animal Ethical Committee of the First People's Hospital of Wenling (approval no. FPHWL20191002) and conducted according to the guidelines of "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [7].

Laboratory reagents and equipment

The reagents and equipment used, and their suppliers (in parenthesis) were: sodium butyrate (Beijing Kerriki Biotechnology Co. Ltd); Nohoring R (China Novo Nord Pharmaceutical Co. Ltd); Insulin ELISA kit (Shanghai Lianmai Engineering Co. Ltd); triglyceride kit (Wuhan Pure Biotechnology Co. Ltd); total cholesterol kit (Wuhan Pure Biotechnology Co. Ltd.); blood sugar meter (Wuhan Pure Biotechnology Co. Ltd); 20 ℃ low temperature refrigerator (Haier); -70 ℃ ultra-low temperature refrigerator (Senxi Technology Co. Ltd); desktop freezing centrifuge

(Beijing Taizejia Technology Development Co. Ltd.); heating agitator (Shanghai Bioengineering Co. Ltd); constant temperature water bath rocker (Shanghai Fuze Trading Co. Ltd); refrigerated centrifuge (Sichuan Shuke Instrument Co. Ltd); whirlpool mixer (Beijing Xinhua Green Source Technology Co. Ltd.); enzyme scale (Beijing Anmai Trading Co. Ltd), and Gene UV spectrophotometer (Dongguan Spectral Standard Experimental Equipment Technology Co. Ltd).

Animal grouping and treatments

Three groups of female mice were used: control, high-fat feed and sodium butyrate groups, with 40 mice in each group. Mice in the control group were given normal diet (60 % carbohydrate, 26 % protein and 14 % fat), while mice in the high-fat diet group received high-fat feed containing 40 % carbohydrate, 20 % protein and 40 % fat. Mice in the sodium butyrate group were given high fat diet and sodium butyrate (95 % high fat diet and 5 % ammonium butyrate).

Measurement of biochemical indices

Blood glucose

Following overnight fast, blood glucose levels of the female mice were determined, once every three weeks, using a glucometer. Blood was obtained by cutting the tail of each mouse.

Serum indicator monitoring: At 12 weeks, following overnight fasting, 50 μL of blood collected from the retrobulbar venous plexus was centrifuged for 10 min at 3000 rpm at 4 ℃. The serum samples obtained from the various groups were kept frozen at -70 ℃ for use in insulin assay with ELISA. After a one-week rest, the female mice were caged every evening with 8-10-week-old male C57BL/6J mice in a ratio of 2:1. The period from the evening to 12 am on the next morning was regarded as 0.5 (12 h) day of pregnancy. On day 14.5 of gestation, blood was collected from the hearts of mice after a 12 h fast, centrifuged for 10 min at 3000 rpm, and the serum samples were kept frozen at -70 ℃ in a refrigerator.

Insulin expression, islet β-cell area and islet β-cell mass

After heart blood collection, pancreatic tissue was rapidly excised, weighed, quickly embedded in OCT, and sliced into 5 μm-thick sections using a frozen microtome. The sections were stored at -20 ℃. One piece was selected from every 6-7 sections to avoid choosing similar parts. Sections from each group were washed thrice with PBS, 5

min for each wash. The tissues were fixed in cold acetone at 4 °C for 10 min, followed by rinsing thrice with PBS. Then, they were treated with lemon acid solution for 25 min at 95 °C for antigen recovery, and washed thrice with PBS, followed by addition of 0.01% Triton X-100 (100 µL, pH 7.4) for 30 min to permeabilize the membranes. The tissues were rinsed thrice with PBS and incubated with dilute horse serum (1:10) at 37 °C for 30 min, followed by incubation overnight at 4 °C with 100 µL of insulin antibody (1:20 dilution). Thereafter, the tissues were incubated with donkey anti-sheep secondary antibody labeled with FIFT (1:100 dilution) at 37 °C for 60 min. The secondary antibody was discarded, and the tissues were stained with DAPI (nucleus stain; 1:1000 dilution) for 1 min, after which the slides were rinsed thrice with PBS, each for 10 min. Then, the slides were treated with fluorescence quenching agent, and observed and photographed under Olympus fluorescence microscope. Images were analyzed with Image-Pro Plus software to measure the fluorescence intensity of the target area. Five islets were randomly selected from each slice for measurement of fluorescence gray value at ×100 magnification. The mean of about 100 islets in each group was obtained at ×400 magnification by measuring the islet area, and the number of positive DAPI staining area in the islet was calculated, i.e., the number of nuclei. The β-cell area was equal to the islet positive staining area/the number of nuclei. At ×400 magnification, β-cell area/pancreatic area was calculated as shown in Eq 1.

$$B = (Aips \times 100)/Pa \dots\dots\dots (1)$$

where $β$ is β-cell area/pancreatic area, $Aips$ is area of insulin positive stain, and Pa is Pancreatic area (with DAPI staining). Islet beta cell mass was calculated as stated in Eq 2.

$$Ibcm = (Ispa \times pm)/Pa \dots\dots\dots (2)$$

where $Ibcm$ is islet beta cell mass, $Ispa$ is insulin staining positive area, while pm is pancreatic mass.

The % apoptosis of islet β cells was determined at x400 magnification. Five (5) islet cells were randomly selected from each slide. The number of DAPI-positive areas in islet was counted with Image-Pro Plus software, and the number of nuclei in each islet was colored red by fluorescence. Bcl-2 positive ratio in each islet cell was calculated using Eq 3.

$$Bpr = Nnrfc/Nigcia \dots\dots\dots (3)$$

where Bpr is Bcl-2 positive ratio, $Nnrfc$ is number of nuclear red fluorescent cells and $Nigcia$ is number of islets in green-colored islet area. About 60 - 80 islets were counted in each group.

Statistical analysis

Measured data are presented as mean ± SD, and F test was employed in determination of statistical differences among the three groups. All statistical analyses were done with SPSS20.0 software. Values of $p < 0.05$ indicated statistically significant differences.

RESULTS

Effect of sodium butyrate on glucose metabolism in obese pregnant mice

Fasting blood glucose concentration in high-fat diet group and sodium butyrate group increased with time, and was higher than that in control, except for week 0. However, the fasting blood glucose in butyrate-treated mice was markedly lower than that in mice given high-fat diet. At 12 weeks and 14.5 weeks of pregnancy, there were markedly higher insulin levels in high-fat diet group and Na butyrate-treated mice than in control mice, but insulin level in sodium butyrate-exposed mice was significantly decreased, relative to the high-fat-fed mice ($p < 0.01$). Concentrations of total cholesterol (TC) and triglycerides (TGs) were markedly higher in the high-fat-fed mice than in control mice, but were markedly lower in the sodium butyrate group. These results are shown in Table 1 and Table 2.

Table 1: Fasting blood glucose levels of female mice (mean ± SD)

Group	Fasting blood glucose （mg/dL）				
	0 week	*3 weeks*	*6 weeks*	*9 weeks*	*12 weeks*
Control	43.16±5.16	43.12±5.16	44.18±4.68	42.75±6.31	45.21±5.23
High-fat diet	45.32±4.89[a]	68.39±10.45[a]	81.54±5.16[a]	101.35±6.37[a]	124.28±7.54[a]
Sodium butyrate	44.21±6.29[ab]	51.26±12.51[ab]	56.89±8.34[ab]	68.39±8.33[ab]	79.35±7.24[ab]
F	1.55	68.30	366.680	691.370	1380.320
P-value	0.215	<0.001	<0.001	<0.001	<0.001

[a]$P < 0.05$, vs control; [b]$p < 0.05$, vs high-fat diet mice

Table 2: Insulin levels in female mice in the three groups (mean ± SD)

Group	Insulin (ng/mL)	
	12 weeks	*14.5 weeks*
Control	0.25±0.02	0.26±0.03
High-fat diet	1.53±0.42[a]	1.76±0.57[a]
Sodium butyrate	0.56±0.34[ab]	0.69±0.26[ab]
F	183.000	181.990
P-value	<0.001	<0.001

[a]$P < 0.05$, vs control; [b]$p < 0.05$, vs high-fat mice

Effect of sodium butyrate on lipid metabolism in obese pregnant mice

As shown in Table 3, TGs and TC levels were markedly raised in mice fed high-fat feed than in control mice, but they were markedly lower in the sodium butyrate group than in high-fat diet-fed mice.

Table 3: Triglyceride and total cholesterol levels in the three groups of female mice (mean ± SD)

Group	Triglycerides	Total cholesterol
Control	142.31±18.25	236.49±41.73
High-fat diet	235.25±29.34[a]	356.43±52.19[a]
Sodium butyrate	175.49±12.43[ab]	244.43±25.18[b]
F	197.332	105.871
P	<0.001	<0.001

[a]$P < 0.05$, vs control; [b]$p < 0.05$, vs high-fat diet mice

Effect of sodium butyrate on insulin expression in islet β cells in obese pregnant mice

Insulin levels were markedly lower in high-fat diet group and sodium butyrate group than control group value, but was markedly raised in the sodium butyrate-treated mice, relative to the high-fat diet mice ($p < 0.01$). Compared to control, there were higher cell area and islet β-cell mass in mice given high fat feed. However, there was higher islet β-cell mass in sodium butyrate group than in control mice ($p < 0.01$). These results are shown in Table 4.

Table 4: Insulin levels, islet β-cell area and islet β-cell mass

Group	Insulin expression	Islet β Cell Area	Islet β Cell Mass
Control	0.18±0.05	90.15±63.33	1.95±0.12
High fat diet	0.04±0.01[a]	112.46±7.52[a]	2.69±0.18[a]
Sodium butyrate	0.11±0.04[ab]	94.26±4.23[b]	2.18±0.16[ab]
F	140.001	130.674	237.732
P-value	<0.001	<0.001	<0.001

Data are presented as mean ± SD. [a]$P < 0.05$, vs control; [b]$p < 0.05$, vs high-fat diet mice

Effect of sodium butyrate on β-cell apoptosis in obese pregnant mouse model

There was markedly higher percentage expression of Bcl-2 in islet β cells in high fat diet-fed mice than in control and sodium butyrate groups ($p < 0.01$). However, Bcl-2 expression level was comparable between sodium butyrate group and control. These data are presented in Figure 1.
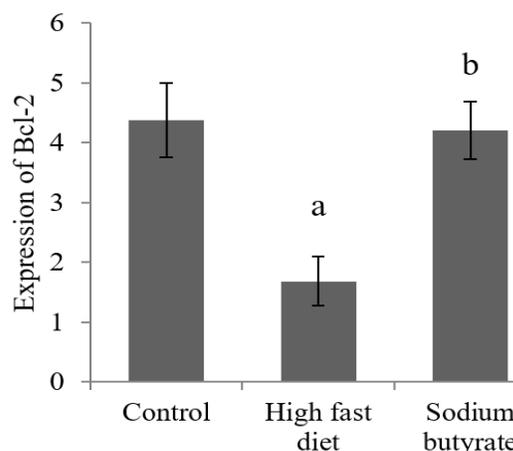
Figure 1: Levels of Bcl-2 expression in the three groups of female mice. Data are presented as mean ± SD. [a]$P < 0.05$, vs control; [b]$p < 0.05$, vs high-fat diet mice

DISCUSSION

At present, obesity and diabetes are widespread in the world, resulting not only in heavy mental and economic burden to patients and their families, but also huge medical and economic burden. Weight loss effectively mitigates insulin resistance. However, for pregnant women, there are few effective and lower-side effect drugs [8-10]. Sodium butyrate, a histone deacylase inhibitor, dissociates DNA from histone octamer after acetylation so that various transcription factors and synergistic transcription factors can bind to DNA specifically and activate gene transcription. After the various treatments, the levels of fasting blood glucose, insulin, triglycerides and TC were markedly raised in high-fat diet mice, relative to control, but were significantly lower in the sodium butyrate group than in high-fat diet group. This suggests that sodium butyrate reduces fasting blood glucose, insulin, triglycerides and total cholesterol levels, and improves glucose and lipid metabolism in obese pregnant rats.

Islet β-cell injury is critical in the etiology T1DM. Glycolipid toxicity, islet amyloid polypeptide deposition, immune cell damage, and

inflammatory response indicate that T2DM is a disease in which islet β-cell quality is reduced and/or its function is abnormal, and insulin secretion is insufficient to meet the normal metabolic needs of the body [11-13]. Therefore, rapid and effective repair of islet β cells, recovery of islet function, and inhibition of islet β cell apoptosis are important in the treatment of GDM and T2DM. Apoptosis of islet β cells is an important cause of diabetes. The quality of islet β cells is derived from increases in cell volume and number of islet β-cells. The number of islet β-cells depends on the balance between islet cell proliferation and apoptosis [14,15]. It is known that Bcl-2 as an anti-apoptotic marker used to study the influence of Na butyrate on the proliferation and anti-apoptotic function of islet β-cells. In this study, insulin expression was markedly lower in high-fat diet mice than in controls, but it was markedly higher in sodium butyrate group than in high-fat diet group, while β-cell area and β-cell quality of islet were markedly higher in mice given high-fat feed than those in control. Moreover, bcl-2 expression level was markedly up-regulated in islet β-cells of high-fat feed group than in control and sodium butyrate groups, but bcl-2 expression level of islet β-cells was comparable between the sodium butyrate group and control group. These results indicate that Na butyrate reduces the β cell area and the β cell quality of islet, improves insulin expression and islet β-cell function, and reduces apoptosis of islet β cells. These findings are similar to results obtained in previous studies.

CONCLUSION

Sodium butyrate significantly reduced levels of fasting blood glucose, insulin, triglycerides and total cholesterol; improved glucose and lipid metabolism, reduced islet β-cell area and islet β-cell quality, increased insulin expression, improved islet β cell function, and effectively inhibited islet β-cell apoptosis in obese pregnant mice.

DECLARATIONS

Acknowledgement

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Zhenzhen Dai designed the study, supervised the data collection, and analyzed the data. Lihong An, Youlin Yang, Yi Chen and Xiahui Zhang interpreted the data and prepared the manuscript for publication. Haiyan Zhang supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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