Research Article

Berberine Attenuates Hyperglycemia by Inhibiting the Hepatic Glucagon Pathway in Diabetic Mice

Ying Zhong, Jing Jin, Peiyu Liu, Yu Song, Hui Zhang, Liang Sheng, Huifang Zhou, and Bijie Jiang

1Department of Pharmacology, School of Basic Medical Science, Nanjing Medical University, 101 Longmian Rd, Nanjing, Jiangsu 211166, China
2Department of Gynecology, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu 210029, China
3Pharmacy College, Xinxiang Medical University, 601 Jinsui Avenue, Xinxiang, Henan 453003, China
4Key Laboratory of Rare Metabolic Diseases, Nanjing Medical University, 101 Longmian Rd, Nanjing, Jiangsu 211166, China
5School of Public Health, Xinxiang Medical University, 601 Jinsui Avenue, Xinxiang, Henan 453003, China

Correspondence should be addressed to Liang Sheng; lgsheng@njmu.edu.cn, Huifang Zhou; zhouhuifang2011301@163.com, and Bijie Jiang; jiangbijie1001@163.com

Received 23 April 2019; Revised 5 September 2019; Accepted 8 October 2019; Published 3 January 2020

Academic Editor: Paola Rizzo

Copyright © 2020 Ying Zhong et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Dysregulated glucagon drives hyperfunction in hepatic glucose output, which is the main cause of persistent hyperglycemia in type 2 diabetes. Berberine (Zhang et al., 2010) has been used as a hypoglycemic agent, yet the mechanism by which BBR inhibits hepatic gluconeogenesis remains incompletely understood. In this study, we treated diabetic mice with BBR, tested blood glucose levels, and then performed insulin, glucose lactate, and glucagon tolerance tests. Intracellular cAMP levels in hepatocytes were determined by ELISA, hepatic gluconeogenetic genes were assayed by RT-qPCR, and the phosphorylation of CREB, which is the transcriptional factor controlling the expression of gluconeogenetic genes, was detected by western blot. BBR reduced blood glucose levels, improved insulin and glucose tolerance, and suppressed lactate- and glucagon-induced hepatic gluconeogenesis in ob/ob and STZ-induced diabetic mice. Importantly, BBR blunted glucagon-induced glucose production and gluconeogenic gene expression in hepatocytes, presumably through reducing cAMP, which resulted in the phosphorylation of CREB. By utilizing a cAMP analogue, adenylate cyclase (AC), to activate cAMP synthetase, and an inhibitor of the cAMP degradative enzyme, phosphodiesterase (PDE), we revealed that BBR accelerates intracellular cAMP degradation. BBR reduces the intracellular cAMP level by activating PDE, thus blocking activation of downstream CREB and eventually downregulating gluconeogenic genes to restrain hepatic glucose production.

1. Introduction

Berberine (BBR), an isoquinoline-type alkaloid originally isolated from Coptis chinensis with a long history of Chinese medicinal application, has been shown to reduce blood glucose levels in diabetes [1, 2]. Hyperglycemia of diabetic patients is largely caused by atherogenic glucose production in the liver [3]. The hypoglycemic effect of BBR is due to its inhibition of hepatic gluconeogenesis [4, 5].

Previous views that BBR downregulates hepatic gluconeogenesis via activation of adenosine monophosphate-activated protein kinase (AMPK) have been challenged by recent investigations that confirmed that AMPK is not necessary or at least not essential for BBR to regulate hepatic gluconeogenesis [6–9]. Thus, how berberine downregulates gluconeogenesis remains unclear.

Hepatic gluconeogenesis is physiologically initiated by glucagon, which activates adenylyl cyclase (AC) to increase the cytosol cyclic AMP (cAMP) level via its receptor on the hepatocyte plasma membrane. cAMP stimulates PKA to phosphorylate cyclic AMP response element binding (CREB), a transcriptional factor that regulates gluconeogenic genes such as phosphoenolpyruvate carboxykinase (Pepck) and glucose-6-phosphatase (G6pc), and thus
increases gluconeogenesis flux \[10-12\]. An abnormally elevated glucagon level and increased hepatic glucagon sensitivity are the primary reasons for hyperglycemia in type 2 diabetic patients \[13, 14\]. Therefore, a target that is commonly used for diabetic therapy is the glucagon signaling pathway in hepatocytes.

In the present study, it was confirmed that BBR targets the glucagon signaling pathway. BBR decreases glucagon-stimulated cAMP levels by activating phosphodiesterase (PDE), the catabolic enzyme of cAMP, which then inhibits hepatic gluconeogenesis. These molecular mechanisms by which BBR operates might provide new strategies to prevent diabetes and related metabolic complications.

2. Materials and Methods

2.1. Animal Experimental Procedures and Materials. All mice were maintained in a temperature-controlled \((22 \pm 2 ^\circ\text{C})\) environment with a 12 h light/dark cycle with free access to standard laboratory chow and water. The animal husbandry and experimental procedures complied with the guidelines of the Animal Care and Ethical Committee of Nanjing Medical University.

The ob/ob mice were purchased at the age of 15 weeks from the Animal Core Facility of Nanjing Medical University. According to procedures from previous reports \[15, 16\], the mice were randomized to two groups to receive berberine (BBR, 5 mg/kg/day, Sigma-Aldrich, St. Louis, MO) or saline (control) intraperitoneally while consuming a normal chow diet. Following a 3-week treatment, the mice were subjected to analysis of blood glucose levels (fasted and fed) and insulin levels in plasma, insulin tolerance test (ITT), glucose tolerance test (GTT), and glucagon (Sigma) and lactate (Sigma) tolerance tests.

Male C57Bl/6j mice at the age of 10 weeks were purchased from the Animal Core Facility of Nanjing Medical University. According to protocols from previous reports \[17-19\], mice were fasted overnight and intravenously injected via the tail with 100 mg/kg streptozotocin (STZ) (Sigma) dissolved in saline. One week after STZ injection, the diabetic mice were randomly divided into two groups: saline-treated (STZ) and BBR (5 mg/kg/day)-treated intraperitoneally while consuming a normal chow diet. Age-matched mice without STZ treatment receiving saline served as the normal group. After a 3-week treatment, the mice were subjected to analysis of blood glucose levels in fasted states and a GTT.

2.2. In Vivo Metabolic Assays. For the GTT and lactate tolerance test, the mice were fasted for 16 h, and then, 0.5 g/kg of glucose or lactate was administered by intraperitoneal injection. For the ITT or glucagon tolerance test, the mice were injected intraperitoneally with 4 U/kg of insulin (Humulin-R; Eli Lilly, Indianapolis, IN) or intravenously with 6 μg/kg of glucagon after fasting for 5 or 15 h, respectively. Blood glucose was measured using an Xceed glucometer (Abbott Diabetes Care, UK), and plasma insulin was measured by an enzyme-linked immunosorbent assay (ELISA) (Millipore, Temecula, CA).

2.3. Primary Hepatocyte Culture. Primary hepatocytes were isolated from male C57Bl/6j mice using a collagenase perfusion as reported previously \[20\]. The cells were cultured in Williams’ E medium (Sigma) supplemented with 6% fetal bovine serum (FBS) (Lonsa, Richmond, VA) and 1% penicillin/streptomycin (Hyclone, Utah) at 37°C in a humidified incubator under an atmosphere of 5% CO₂-95% air.

2.4. Glucose Production in Hepatocytes. Once the hepatocytes were attached, the medium was switched to serum-free Williams’ E medium for further culture for 5 hours. Then, cells were cultured in serum-free/glucose-free/phenol red-free Dulbecco’s modified Eagle’s medium supplemented with 15 mM lactate while being treated with glucagon (50 nM, Sigma) or BBR (10 μM). Four hours later, the medium was used for the glucose assay by employing a Glucose Assay Kit (Jiancheng, Nanjing, China). Hepatocytes were lysed for the protein assay, which was performed using a Bradford protein assay kit (Jiancheng). The glucose production was calculated as the amount of glucose in medium normalized by the amount of protein in one hour.

2.5. cAMP Measurement. cAMP levels in hepatocytes and the liver were analyzed using a colorimetric cAMP ELISA kit (Cell Biols, San Diego, CA), according to the manufacturer’s instructions. Briefly, hepatocytes in 12-well plates were incubated in serum-free Williams’ E medium for 4 h and subjected to 10 μM BBR for 15 min, followed by 50 nM glucagon for 10 min. Hepatocytes were lysed by 0.1 M HCl. cAMP concentrations were normalized to total cellular protein. ob/ob mice were fasted and treated with saline or 5 mg/kg body weight of BBR. One hour later, mice were injected intravenously with 15 μg/kg glucagon, and livers were collected 15 min later.

2.6. Western Blotting. Extracts of the liver and hepatocytes prepared by RIPA buffer were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). The blots were detected by the primary antibodies against CREB (Abclonal, A11989, 1:1000), phosho-CREB (S133) (Abcam, ab32096, 1:1000), Lamin B1 (CST, 13435, 1:1000), and GAPDH (CST, 13384, 1:1000).

2.7. Quantitative Real-Time PCR. Total RNA was isolated from the liver and hepatocytes using RNAiso Plus (Takara, Beijing, China) according to the manufacturer’s instructions. cDNA was synthesized from 2.0 μg total RNA using the 5x All-In-One RT MasterMix (abm, Richmond, BC), and Real-time PCR (qPCR) was performed using SYBR Green qPCR Master Mix (Bimake, Houston, TX) with an ABI 7900 RT-PCR machine. The primer sets used were as follows: phosphoenolpyruvate carboxykinase (Pepck) forward (5′-TCAGCTGAACGGTCTGG-3′) and Pepck reverse (5′-GCTTTTCTCAAGGTCTCCT-3′), glucose-6-phosphatase (G6pc) forward (5′-GAGAGAAGGATGAA CATTCT-3′) and G6pc reverse (5′-GGTCGGTGTCACCA
GGTGAC-3′), and 36B4 forward (5′-AAGCGCGTCTCGGCATTGTCT-3′) and 36B4 reverse (5′-CGGCAGGGCCAGCA GTGGT-3′). The expression level of each gene was normalized to the expression of 36B4.

2.8. Statistical Analysis. The data are expressed as the mean ± standard error (S.E.). The data between groups were analyzed by ANOVA (GraphPad Prism, La Jolla, CA) or Student’s t-test. P < 0.05 was considered statistically significant.

3. Results

3.1. Berberine Improves Glucose Metabolism in Diabetic Mice. The leptin-deficient mouse (ob/ob), which is the
classical type 2 diabetic model, and the STZ-treated mouse, which is the classical type 1 diabetic model, were used to test the hypoglycemic effect of BBR. Others have previously identified that BBR may interact with the gut microbiota, and this important mechanism may prevent obesity and insulin resistance [21–23]. Therefore, we changed the administration of BBR from gavage to intraperitoneal. The results before the changes are presented in Supplementary Figs. 1 and 2.

The levels of fed blood glucose and insulin in ob/ob mice were significantly decreased by BBR treatment, which also reduced the fasting blood glucose level but did not reach significant difference (Figures 1(a) and 1(b)). The glucose and insulin tolerance in BBR-treated ob/ob mice was significantly improved compared to those in control mice (Figures 1(c) and 1(d)).

STZ successfully provoked hyperglycemia, and BBR treatment significantly decreased fasting blood glucose (Figure 1(e)) and improved glucose intolerance (Figure 1(f)) in STZ-induced diabetic mice, suggesting that berberine decreased hyperglycemia in an insulin-independent manner.

3.2. Berberine Decreased Hepatic Gluconeogenesis in the Livers of Diabetic Mice. To test the effects of BBR on hepatic gluconeogenesis, we subjected the animals to BBR and lactate, a gluconeogenetic substrate. The hyperglycemia in response to lactate was blunted after BBR treatment in ob/ob (Figure 2(a)) and STZ-induced diabetic mice (Figure 2(c)). Moreover, the mRNA levels of gluconeogenic genes Pepck, G6pc, and PGC1α were downregulated by BBR in the livers of ob/ob (Figure 2(b)) and STZ-induced diabetic mice (Figure 2(d)). These data suggest that BBR reduces hepatic glucose production by downregulating the gluconeogenic genes.

3.3. Berberine Suppressed Glucagon-Induced CREB Phosphorylation and Hepatic Glucose Production. CREB, the main transcriptional factor controlling the expression...
of gluconeogenic genes in the liver, is activated by phosphorylation via the glucagon/cAMP/PKA pathway [24]. To examine whether BBR regulates the expression of gluconeogenic genes by affecting CREB activity, we utilized primary hepatocytes treated with BBR and/or glucagon as the inspection platform.

BBR reduced the basal and glucagon-induced glucose production (Figure 3(a)) as well as the mRNA levels of 

of gluconeogenic genes in the liver, is activated by phosphorylation via the glucagon/cAMP/PKA pathway [24]. To examine whether BBR regulates the expression of gluconeogenic genes by affecting CREB activity, we utilized primary hepatocytes treated with BBR and/or glucagon as the inspection platform.

BBR reduced the basal and glucagon-induced glucose production (Figure 3(a)) as well as the mRNA levels of 

butyl-1-methylxanthine (IBMX). We found that BBR inhibited 8-bromo-cAMP (Figure 4(c)) and forskolin-stimulated (Figure 4(d)) phosphorylation of CREB but could not reverse IBMX-stimulated (Figure 4(e)) phosphorylation, suggesting that BBR may accelerate cAMP degradation by activating PDE.

3.4. Berberine Reversed the Glucagon-Induced Increase in Intercellular cAMP Levels. To determine whether BBR inhibits the phosphorylation of CREB via decreasing the cAMP level, we measured cAMP levels in hepatocytes and the liver. The results showed that glucagon dramatically increased cAMP levels in hepatocytes and the liver, and they were significantly reduced by BBR (Figures 4(a) and 4(b)), suggesting that cAMP is involved in the regulation of CREB phosphorylation by BBR. To lock the action target of BBR in the glucagon pathway, we detected the phosphorylation of CREB in response to 8-bromo-cAMP, forskolin, and 3-isobutyl-1-methylxanthine (IBMX). We found that BBR inhibited 8-bromo-cAMP (Figure 4(c)) and forskolin-stimulated (Figure 4(d)) phosphorylation of CREB but could not reverse IBMX-stimulated (Figure 4(e)) phosphorylation, suggesting that BBR may accelerate cAMP degradation by activating PDE.

4. Discussion

To relieve hyperglycemia in type 2 diabetes, the predominant therapeutic strategy is to suppress hepatic gluconeogenesis [9, 25]. The present study reveals that BBR attenuates hepatic gluconeogenesis by blocking the glucagon pathway, the main hormone that increases blood glucose.

A variety of studies have demonstrated that BBR attenuates hepatic gluconeogenesis and hyperglycemia by relieving insulin resistance in type 2 diabetes [26–28], which is consistent with the enhanced insulin sensitivity and reduced glucose levels observed in ob/ob mice treated with BBR in the present investigation. However, a hypoglycemic effect caused by BBR was also observed in mice lacking insulin due to the STZ-induced destruction of pancreatic ß-cells. This suggests that in addition to increasing insulin sensitivity, BBR exerts a hypoglycemic effect in an insulin-independent manner.
The glucagon pathway is suspected of being the target of BBR beyond the insulin pathway because abnormally elevated glucagon levels and increased hepatic glucagon sensitivity drive hepatic gluconeogenesis in type 2 diabetic patients [13]. Indeed, BBR reduced the amount of lactate and glucagon-triggered hepatic glucose production and downregulated gluconeogenetic genes including Pepck, G6pc, and PGC-1α in the liver and hepatocytes. CREB, the transcriptional factor regulating the gluconeogenetic genes above, is located downstream of the glucagon signaling pathway. It is activated by phosphorylation induced via the glucagon/glucagon receptor/AC/cAMP/PKA pathway [9, 29]. The level of cAMP, a second messenger transferring signals of glucagon into cells, is controlled by multiple nodes, including cAMP synthetase (AC) and cAMP degrading enzyme (PDE) [30, 31]. We found that BBR inhibited CREB phosphorylation via reducing the level of cAMP in the cytoplasm of hepatocytes. Furthermore, BBR downregulates cAMP by activating PDE. Actually, PDE breaks the phosphodiester bond in cAMP and regulates the localization, duration, and
amplitude of cAMP signaling within subcellular domains. PDEs are therefore important regulators of signal transduction mediated by these second messenger molecules.

In conclusion, BBR accelerates intracellular cAMP degradation by activating PDE and thus blocks the hepatic gluconeogenesis pathway through downregulating CREB phosphorylation and the downstream gluconeogenic genes, thereby reducing blood glucose in mice. Our data indicate that BBR plays a pivotal role as a regulator of gluconeogenesis in the diabetic state.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors’ Contributions

Ying Zhong and Jing Jin contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 81770862, 81602828, and 81774354), the Leading Talents of Chinese Medicine in Jiangsu Province (No. SLJ0202), the Science and Technology Development Foundation from Nanjing Medical University (No. 2017N)MU015), the Higher Education Key Research Project of Henan Province Education Department (No. 15A310008), the Support Project for the Disciplinary Group of Psychology and Neuroscience, Xinxiang Medical University, and the Henan Collaborative Innovation Center of Molecular Diagnosis and Laboratory Medicine to Liang Sheng or Bijie Jiang.

Supplementary Materials

Supplementary Fig. 1: BBR improves glucose metabolism in ob/ob mice. The mice were gavaged with saline (control) or BBR once a day for 3 weeks while consuming a normal chow diet. (A) Feeding and fasting blood glucose. (B) Fasting plasma insulin levels. (C) Glucose tolerance test. (D) Insulin tolerance test. (E) Lactate tolerance test. (F) Glucagon tolerance test. Areas under the curve (AUCs) were calculated. Each value represents the mean ± S.E. *P < 0.05 compared with the control group (N = 7-8). Supplementary Fig. 2: BBR improves glucose metabolism in STZ-induced diabetic mice. The mice were gavaged with saline (normal), and the STZ-induced mice were treated with saline (STZ) or BBR (STZ+BBR) once a day for 3 weeks while consuming a normal chow diet. (A) Fasting blood glucose and 12 hr AUCs of the STZ and BBR groups. (B) Glucose tolerance test. (C) Lactate tolerance test. Areas under the curve (AUCs) were calculated. Each value represents the mean ± S.E. *P < 0.05 compared with the STZ group (N = 7-8).

References

[1] H. Zhang, J. Wei, R. Xue et al., “Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression,” *Metabolism*, vol. 59, no. 2, pp. 285–292, 2010.

[2] J. Yin, H. Xing, and J. Ye, “Efficacy of berberine in patients with type 2 diabetes mellitus,” *Metabolism*, vol. 57, no. 5, pp. 712–717, 2008.

[3] R. A. Rizza, “Pathogenesis of fasting and postprandial hyperglycemia in type 2 diabetes: implications for therapy,” *Diabetes*, vol. 59, no. 11, pp. 2697–2707, 2010.

[4] M. Zhang, X. Lv, J. Li et al., “Saccharate caprates augments the hypoglycemic effect of berberine via AMPK in inhibiting hepatic gluconeogenesis,” *Molecular and Cellular Endocrinology*, vol. 363, no. 1-2, pp. 122–130, 2012.

[5] B. Zhang, Y. Pan, L. Xu et al., “Berberine promotes glucose uptake and inhibits gluconeogenesis by inhibiting deacetylase SIRT3,” *Endocrine*, vol. 62, no. 3, pp. 576–587, 2018.

[6] X. Xia, J. Yan, Y. Shen et al., “Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis,” *PloS One*, vol. 6, no. 2, p. e16556, 2011.

[7] S. J. Jiang, H. Dong, J. B. Li et al., “Berberine inhibits hepatic gluconeogenesis via the LKB1-AMPK-TORC2 signaling pathway in streptozotocin-induced diabetic rats,” *World Journal of Gastroenterology*, vol. 21, no. 25, pp. 7777–7785, 2015.

[8] M. Foretz, S. Hébrard, J. Leclerc et al., “Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state,” *The Journal of Clinical Investigation*, vol. 120, no. 7, pp. 2355–2369, 2010.

[9] R. A. Miller, Q. Chu, J. Xie, M. Foretz, B. Voilet, and M. J. Birnbaum, “Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP,” *Nature*, vol. 494, no. 7436, pp. 256–260, 2013.

[10] J. C. Chrvia, R. P. S. Kwock, N. Lamb, M. Hagiwara, M. R. Montminy, and R. H. Goodman, “Phosphorylated CREB binds specially to the nuclear protein CBP,” *Nature*, vol. 365, pp. 855–859, 1993.

[11] G. A. Gonzalez and M. R. Montminy, “Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133,” *Cell*, vol. 59, no. 4, pp. 675–680, 1989.

[12] G. Jiang and B. B. Zhang, “Glucagon and regulation of glucose metabolism,” *American Journal of Physiology-Endocrinology and Metabolism*, vol. 284, no. 4, pp. E671–E678, 2003.

[13] D. D’Alessio, “The role of dysregulated glucagon secretion in type 2 diabetes,” *Diabetes, Obesity and Metabolism*, vol. 13, pp. 126–132, 2011.

[14] L. Sheng, Y. Zhou, Z. Chen et al., “NF-kB-inducing kinase (NIK) promotes hyperglycemia and glucose intolerance in obesity by augmenting glucagon action,” *Nature Medicine*, vol. 18, no. 6, pp. 943–949, 2012.

[15] Z. Zhang, H. Zhang, B. Li et al., “Berberine activates thermogenesis in white and brown adipose tissue,” *Nature Communications*, vol. 5, no. 1, 2014.

[16] H. W. Jeong, K. C. Hsu, J.-W. Lee et al., “Berberine suppresses proinflammatory responses through AMPK activation in macrophages,” *American Journal of Physiology-Endocrinology and Metabolism*, vol. 296, no. 4, pp. E955–E964, 2009.

[17] M. S. Islam and T. du Loo, “Experimental rodent models of type 2 diabetes: a review,” *Methods and Findings in
Experimental and Clinical Pharmacology, vol. 31, no. 4, pp. 249–261, 2009.

[18] M. S. Islam and R. D. Wilson, “Experimentally induced rodent models of type 2 diabetes,” Methods in Molecular Biology, vol. 933, pp. 161–174, 2012.

[19] A. Ghasemi, S. Khalifi, and S. Jedi, “Streptozotocin-nicotinamide-induced rat model of type 2 diabetes (review),” Acta Physiologica Hungarica, vol. 101, no. 4, pp. 408–420, 2014.

[20] P. Li, D. Y. Oh, G. Bandyopadhyay et al., “LTB4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes,” Nature Medicine, vol. 21, no. 3, pp. 239–247, 2015.

[21] Y. Guo, Y. Zhang, W. Huang, F. P. Selwyn, and C. D. Klæsens, “Dose-response effect of berberine on bile acid profile and gut microbiota in mice,” BMC Complementary and Alternative Medicine, vol. 16, no. 1, 2016.

[22] Y. Wang, Q. Tong, J. W. Shou et al., ”Gut microbiota-mediated personalized treatment of hyperlipidemia using berberine,” Theranostics, vol. 7, no. 9, pp. 2443–2451, 2017.

[23] X. Zhang, Y. Zhao, M. Zhang et al., ”Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats,” PLoS one, vol. 7, no. 8, 2012.

[24] B. Y. Park, J. H. Jeon, Y. Go et al., ”PDK4 Deficiency Suppresses Hepatic Glucagon Signaling by Decreasing cAMP Levels,” Diabetes, vol. 67, no. 10, pp. 2054–2068, 2018.

[25] L. Ozcan, C. C. L. Wong, G. Li et al., ”Calcium signaling through CaMKII regulates hepatic glucose production in fasting and obesity,” Cell Metabolism, vol. 15, no. 5, pp. 739–751, 2012.

[26] A. Pirillo and A. L. Catapano, ”Berberine, a plant alkaloid with lipid- and glucose-lowering properties: From _in vitro_ evidence to clinical studies,” Atherosclerosis, vol. 243, no. 2, pp. 449–461, 2015.

[27] F. H. Geng, G. H. Li, X. Zhang et al., ”Berberine improves mesenteric artery insulin sensitivity through up-regulating insulin receptor-mediated signalling in diabetic rats,” British Journal of Pharmacology, vol. 173, no. 10, pp. 1569–1579, 2016.

[28] Y. S. Lee, W. S. Kim, K. H. Kim et al., ”Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states,” Diabetes, vol. 55, no. 8, pp. 2256–2264, 2006.

[29] Y. Li, J. Song, Y. Tong, S. K. Chung, and Y. H. Wong, ”RGS19 upregulates Nm23-H12 metastasis suppressors by transcriptional activation via the cAMP-PKA-CREB pathway,” Onco-target, vol. 8, pp. 69945–69960, 2017.

[30] S. Uckert, P. Hedlund, K. E. Andersson, M. C. Truss, U. Jonas, and C. G. Stief, ”Update on Phosphodiesterase (PDE) Isoenzymes as Pharmacologic Targets in Urology: Present and Future,” European Urology, vol. 50, no. 6, pp. 1194–1207, 2006.

[31] M. F. Azevedo, F. R. Fauçz, E. Bimpaki et al., ”Clinical and molecular genetics of the phosphodiesterases (PDEs),” Endocrine Reviews, vol. 35, no. 2, pp. 195–233, 2014.