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Research Paper

Berberine Reduces Pyruvate-driven Hepatic Glucose Production by Limiting Mitochondrial Import of Pyruvate through Mitochondrial Pyruvate Carrier 1

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ABSTRACT

Background: Mitochondrial pyruvate import via mitochondrial pyruvate carrier (MPC) is a central step in hepatic gluconeogenesis. Berberine inhibits hepatic gluconeogenesis, but the mechanism is incompletely understood. This study aims to investigate whether berberine could reduce excessive hepatic glucose production (HGP) by limiting mitochondrial import of pyruvate through MPC1.
Methods: High-fat diet (HFD) feeding augmented HGP. The effects of berberine on hepatic fatty acid oxidation, sirtuin3 (SIRT3) induction and mitochondrial pyruvate carrier 1 (MPC1) function were examined.
Findings: HFD feeding increased hepatic acetyl coenzyme A (acetyl CoA) accumulation with impaired pyruvate dehydrogenase (PDH) activity and increased pyruvate carboxylase (PC) induction. Berberine reduced acetyl CoA accumulation by limiting fatty acid oxidation and prevented mitochondrial pyruvate shift from oxidation to gluconeogenesis through carboxylation. Upon pyruvate response, SIRT3 binded to MPC1 and stabilized MPC1 protein via deacetylation modification, facilitating mitochondrial import of pyruvate. Berberine preserved the acetylation of MPC1 by suppression of SIRT3 induction and impaired MPC1 protein stabilization via protein degradation, resultantly limiting mitochondrial pyruvate supply for gluconeogenesis.
Interpretation: Berberine reduced acetyl CoA contents by limiting fatty acid oxidation and increased MPC1 degradation via preserving acetylation, thereby restraining HGP by blocking mitochondrial import of pyruvate. These findings suggest that limitation of mitochondrial pyruvate import might be a therapeutic strategy to prevent excessive hepatic glucose production.

1. Introduction

Blood glucose levels fluctuate during the feeding and fasting cycles and the glycemic control is maintained within a physiological range by the regulation of glucose production and disposal [1]. While insulin prevents postprandial hyperglycemia by promoting glucose disposal, the glucose homeostasis during fasting is mainly maintained by hepatic gluconeogenesis. However, inappropriate hepatic glucose production (HGP) is a major contributor that results in fasting hyperglycemia, especially in diabetes [2]. The vast majority of gluconeogenic carbon flux is routed through the mitochondrial matrix, while pyruvate is thought to be the major mitochondrially imported substrate for hepatic glucose production [3]. Gluconeogenesis via pyruvate/lactate predominates during prolonged food deprivation and this regulation is deranged in the diabetic liver, contributing to excessive HGP [4]. Therefore, limitation of mitochondrial pyruvate availability for substrate supply should prevent fasting hyperglycemia in diabetes.

Cytoplasmic pyruvate is mainly derived from glycolysis, while systemically produced lactate and alanine are also the sources from different mechanisms. The import of pyruvate into mitochondria is mediated by mitochondrial pyruvate carrier (MPC). MPC is composed of MPC1 and MPC2, which form a hetero-oligomeric complex in the inner mitochondrial membrane, and both proteins are required for the complex

Abbreviations: AMPK, AMP-activated protein kinase; DCA, Dichloroacetate; FACD, Fatty acyl CoA dehydrogenase; G-6-Pase, Glucose-6-phosphatase; HFD, High-fat diet; HGP, Hepatic glucose production; KCT, β-ketoacyl CoA thiolase; LDH, Lactate dehydrogenase; MPC, Mitochondrial pyruvate carrier; NAM, Nicotinamide; NEFAs, Non-esterified free fatty acids; PC, Pyruvic carboxylase; PDH, Pyruvate dehydrogenase; PDK, Pyruvate dehydrogenase kinase; PEPCK, Phosphoenolpyruvate carboxykinase; PGC-1α, Peroxisome proliferator-activated receptor-γ coactivator-1α; SIRT3, Sirtuin3; TC, Total cholesterol; TAC, Tricarboxylic acid cycle; TG, Triglyceride; THP, 3-(2,2,2-Trimethylhydrazine) propionate (also known as mildronate); TMZ, Trimetazidine..

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Research in Context

Evidence before this Study

Mitochondrial pyruvate carrier is composed of MPC1 and MPC2, and the increased MPC1 expression in diabetes is responsible for hyperglycemia. Hepatic gluconeogenesis is accompanied with altered metabolism and redox state, and MPC1 emerges as a key control to regulated pyruvate-driven glucose production in the liver.

Added Value of This Study

Fatty acid load enhanced hepatic fatty acid oxidation with altered redox state. SIRT3 activation improved MPC1 protein stability via deacetylation, and thus increased MPC1 protein function to import pyruvate into mitochondria, ensuring mitochondrial pyruvate' availability for hepatic gluconeogenesis. Berberine limited hepatic fatty acid oxidation via mitochondrial complex I inhibition and suppressed SIRT3 activation via reducing NAD^+/NADH ratio. Berberine impaired MPC1 stability by preserving acetylation, and thereby reduced MPC1 expression through protein degradation. Although other gluconeogenic substrates also increased hepatic glucose production, berberine mainly restrained pyruvate-driven glucose production in a manner dependent on MPC1 inhibition.

Implications of All Available Evidence

Our work suggests that pharmacological inhibition of MPC1 induction by metabolic regulation could reduce excessive hepatic glucose output in diabetes.

stabilize and full activity [5, 6]. Once in mitochondria, pyruvate is either channeled toward carboxylation by the enzyme pyruvate carboxylase (PC) for gluconeogenesis or oxidation by the enzyme pyruvate dehydrogenase (PDH). The role of MPC in mitochondrial pyruvate import is responsible for pancreatic β cell glucose sensing [7]. Thiazolidinediones (TZDs) are the most effective agents for preventing hyperglycemia in type 2 diabetes and unexpectedly found to inhibit MPC in muscle cells in a manner independent of PPARγ [8]. Recently, it is documented that pyruvate-driven hepatic gluconeogenesis is MPC dependent, and liver-specific loss of MPC activity impairs gluconeogenesis and decreases hyperglycemia in obesity and diabetes [9, 10]. Although other substrates, such as alanine and glutamine, can serves as gluconeogenic carbon flux in mitochondrial matrix, independent of MPC transportation [9, 10], it is now generally accepted that mitochondrial pyruvate import via the MPC is a central step in hepatic gluconeogenesis.

Hepatic gluconeogenesis is an anabolic process and needs fatty acid oxidation to supply energy [11]. Fatty acid mobilization increases hepatic fatty acid oxidation, and abnormal fatty acid metabolism has a profound impact on hepatic metabolism, leading to lipid deposition, hepatic insulin resistance and glucose overproduction [12, 13]. However, the potential implication of hepatic fatty acid oxidation in disturbed gluconeogenesis is little known. Adipose lipolysis-derived nonesterified fatty acids (NEFAs) enter into the liver and increase hepatic acetyl CoA to impair insulin sensitivity [12, 13]. More than a metabolic intermediate, acetyl CoA acts as a second messenger to inhibit pyruvate dehydrogenase (PDH) activity [14] and allosterically activate pyruvate carboxylase (PC) which catalyzes the irreversible carboxylation of pyruvate to oxaloacetate [15]. Logically, this regulation should facilitate mitochondrial pyruvate to be rerouted away from oxidation toward carboxylation for gluconeogenesis, establishing the functional link between hepatic fatty acid oxidation and hepatic gluconeogenesis.

Protein lysine acetylation is a key post-translational modification in fuel metabolism. Acetyl CoA is the major donor of the acetyl groups for acetylation and lysine acetyltransferases use acetyl CoA as an essential cofactor to donate an acetyl group to the target lysine residue [16]. In contrast, acetylated proteins could be deacetylated by sirtuins (SIRTs), a family of nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases. Sirtuin 3 (SIRT3) is predominantly localized in the mitochondrial matrix and involved in the metabolic regulation of obesity and diabetes [17, 18]. Although SIRT3 is also shown to deacetylate MPC1 to enhance its activity in cancer cells [19], its action in the regulation of hepatic MPC1 and gluconeogenesis is unknown.

Berberine is an isoquinoline alkaloid isolated from Coptis chinensis and exerts the ability to lower blood glucose in diabetes. Similar to metformin, berberine regulates adenosine monophosphate-activated protein kinase (AMPK) activity, and this action is proposed to inhibit hepatic gluconeogenesis [20–22]. But this concept has been challenged by recently published studies which showed that AMPK is dispensable for the regulation of hepatic gluconeogenesis [23, 24]. Berberine shows anti-hyperlipidemia with reduced hepatic lipid deposition, which has been observed in both animals and human [25, 26], suggesting the possibility that regulation of lipid metabolism by berberine in the liver contributes to restraining HGP. Fatty acid mobilization during fasting and lipid disorders in obese and diabetes increase hepatic fatty acid oxidation to drive gluconeogenesis by raising mitochondrial levels of reducing equivalents and acetyl CoA [27]. Therefore, we hypothesized that improved lipid metabolism and redox homeostasis by berberine should contribute to reducing HGP. In this context, we investigated the effects of berberine on hepatic energy state with focus on the regulation of MPC1 function. We showed that berberine limited fatty acid oxidation and reduced mitochondrial SIRT3 induction, resultantly promoting MPC1 degradation by preserving acetylation. These findings not only identify an unrecognized role of berberine in the regulation of hepatic gluconeogenesis, but also suggest that limitation of mitochondrial pyruvate import might be a therapeutic strategy to reduce excessive hepatic glucose production.

2. Materials and Methods

2.1. Reagents

Berberine hydrochloride (purity ≥ 98%) was obtained from Nantong Jingwei Biological Technology Co, Ltd. Metformin hydrochloride (purity >98%) was purchased from Shanghai Sangon biological engineering Co, Ltd. Resveratrol (purity >98%) was from Nanjing Zelang medical technology co., LTD. Trimetazidine (TMZ) was from British Kinase Chemicals LTD. Mildronate (also known as 3-(2,2,2-Trimethylhydrazine) propionate, THP) was supplied by Dalian Meilun Biological Technology Co, Ltd. Dichloroacetate (DCA) was purchased from Sigma-Aldrich. UK-5099, CPI-613 and nicotinamide (NAM) were from ApexBio Technology. Cycloheximide and MG-132 were from MedChem Express (Shanghai, China). Lactate, alanine, glutamine and methyl pyruvate were from Aladdin (Shanghai, China). Palmitate (PA) was from Sinopharm Chemical Reagent Co, Ltd. (Shanghai, China) and dissolved in ethanol, and then diluted with 10% FFA-free bovine serum albumin (BSA) at the ratio of 1:19 before use.

2.2. Animals

ICR male mice (18–20 g) were purchased from Beijing Vital River Laboratory Animal Technology Co, Ltd. After adaptive feed in 12 h dark-light cycles ad libitum with free access to water and food for a week, mice were randomized to groups to receive berberine (100 mg kg^{-1} day^{-1}) or metformin (200 mg kg^{-1} day^{-1}) by gavage and to receive either a normal chow diet or a high-fat diet (HFD) (10% lard, 10% yolk, 1% cholesterol, 0.2% cholate and 78.8% standard diet) for 10 weeks. All experimental procedures were approved by the Institution

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