β-hydroxybutyric acid as a potential therapeutic metabolite for type 2 diabetes mellitus (Review)

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Abstract. Type 2 diabetes mellitus (T2DM) is a major metabolic disease that poses a threat to human health; therefore, the development of new pharmaceutical therapies for the treatment of T2DM is of great importance. β-hydroxybutyric acid (β-HB) is the primary ketone body present in the human body. β-HB not only serves as an energy substrate to maintain the metabolic homeostasis of the body but also acts as a signaling molecule, exerting multiple biological functions both inside and outside cells. The present review summarizes the research progress and latest findings of β-HB in T2DM models from the perspective of metabolism, physiological effects and potential as a therapeutic agent. Research indicates that β-HB exerts protective effects against T2DM by regulating glucose and lipid metabolism, preserving the integrity of pancreatic β-cells and improving insulin resistance (IR). Additionally, β-HB can alleviate the core pathological conditions of T2DM and related complications by enhancing the stability of cellular proteins, reducing oxidative stress and controlling inflammatory responses and endoplasmic reticulum stress (ERS), while regulating mitochondrial biogenesis, autophagy and apoptosis. Furthermore, the present review also describes the application of β-HB in clinical research on T2DM. Research indicates that regulating β-HB levels through endogenous and exogenous ketogenesis approaches can influence body weight, fasting blood glucose levels, IR and memory ability in T2DM patients. These results suggest that β-HB is a potential metabolite for T2DM treatment.

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Abbreviations: β-HB, β-hydroxybutyric acid; KB, ketone body; KD, ketogenic diet; BDH1, β-hydroxybutyrate dehydrogenase 1; MCTs, monocarboxylate transporters; SMCTs, sodium-dependent MCTs; IF, intermittent fasting; CR, caloric restriction; GPCR, G protein-coupled receptor; Kbhb, lysine β-hydroxybutyrylation; KA, ketoacidosis; NK, nutritional ketosis; DKA, diabetic ketoacidosis; DKD, diabetic kidney disease; 1,3-BDO, 1,3-butanediol; HCAR2, hydroxycarboxylic acid receptor 2; DCM, diabetic cardiomyopathy; DE, diabetic encephalopathy; AGEs, advanced glycation end products; DR, diabetic retinopathy; KE, ketone esters; KME, ketone monoester

Key words: type 2 diabetes mellitus, ketone body, β-hydroxybutyric acid, treatment

Contents

1. Introduction

As reported in the Diabetes Atlas (11th Edition) published by the International Diabetes Federation (2025), 589 million adults around the world are estimated to be living with diabetes mellitus (DM), with forecasts suggesting that this figure could increase to 853 million by 2050 (1). DM is a metabolic condition that results from an interplay of genetic and environmental influences, which can lead to inadequate insulin production and/or impaired insulin function. The condition is primarily manifested by dysregulation in the metabolism of carbohydrates, proteins and fats, and clinically presents as chronic hyperglycemia (2). Until now, the full causes and development of DM have yet to be fully understood. DM is mainly divided into four types: Type 1 DM (T1DM), T2DM, gestational DM and other specific diabetes types not previously enumerated.

Among these, T2DM is the most common type, representing ~90% of all diabetes cases (3); thus, it is the core focus of the present review. T2DM is caused by genetic susceptibility and environmental risk factors, which lead to the inability of β-cells to produce sufficient insulin or the poor effectiveness of insulin (4,5). In terms of genetic factors, T2DM exhibits a significant family aggregation pattern. At present, a number of susceptibility genes (such as transcription factor 7 like 2, peroxisome proliferator-activated receptor γ and potassium inwardly rectifying channel subfamily J member 11) have been identified, which can affect an individual's susceptibility to the disease by regulating processes including insulin secretion, glucose transport and lipid metabolism (6). Among the environmental factors, obesity, a high-calorie diet, lack of physical activity and age are the main factors for T2DM. Additionally, patients with hypertension or dyslipidemia have an increased risk of developing T2DM. Moreover, T2DM can result in chronic metabolic disorders accompanied by multi-system complications, which may lead to the onset of eye disease, kidney disease, cardiac disease, vascular disease and dysfunction of the central nervous system (7).

The present treatment plan for T2DM involves a comprehensive strategy that includes considerable lifestyle changes along with medication interventions. Pharmacological agents mainly consist of oral hypoglycemic medications and insulin formulations. Oral hypoglycemics encompass various medications, including traditional drugs such as metformin, α-glucosidase inhibitors, glinides, sulfonylureas and thiazolidinediones, and newer agents such as dipeptidyl peptidase-4 inhibitors. Traditional oral hypoglycemic medications have been demonstrated to improve glycemic management and decrease the likelihood of complications and mortality associated with T2DM. However, their effectiveness across various organ systems, particularly the cardiovascular and renal systems, is restricted, and they come with specific side effects (7). For instance, α-glucosidase inhibitors cause various side effects including abdominal discomfort, bloating, diarrhea, pain and flatulence (8). Hypoglycemia is the main side effect of all sulfonylurea drugs, while minor side effects such as headache, dizziness, nausea, hypersensitivity reactions and weight gain are also common (9). As a result, reliance on these medications has decreased in preference for newer therapies, such as insulin pumps, sodium-glucose cotransporter-2 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, which have demonstrated notable efficacy. However, the percentage of patients achieving well-controlled T2DM has not risen as expected (10). Insulin treatment encompasses a variety of insulin types, such as basal insulin and premixed (or biphasic) insulin analogs. Commonly utilized basal insulins include neutral protamine hagedorn insulin, insulin glargine in U100 or U300 formulations and detemir (11). At the same time, lifestyle changes, which include dietary adjustments and regular exercise, are essential for the successful management of T2DM and its related complications (12). However, clinical data show that the proportion of T2DM patients with well-controlled blood glucose has not met expectations, suggesting that there is still room for optimization in existing treatment regimens (13).

β-hydroxybutyric acid (β-HB) is the most abundant ketone body (KB) in the human body, accounting for ~70% of the circulating KBs (14). β-HB serves as an efficient energy carrier from the liver to peripheral tissues and it can act as a crucial alternative energy source, especially when glucose supply for energy production is insufficient. The maintenance of its concentration in the body mainly relies on two pathways: i) Endogenous production when the body is in a state of insufficient glucose supply for energy such as prolonged starvation, ketogenic diet (KD) with a low-carbohydrate and high-fat ratio and after strenuous exercise (15); ii) exogenous ketone supplement. Exogenous supplementation can rapidly increase the concentration of β-HB in the circulation (16). Recent studies have shown that β-HB is not merely a metabolite; it also possesses important cellular signaling functions as it can link changes in the external environment to cellular functions and gene expression by regulating key intracellular pathways. Specifically, studies have confirmed that β-HB plays an important role in the pathogenesis and therapeutic management of diseases such as aging (17), intestinal diseases (18), liver diseases (19), septicemia (20), obesity (21) and T2DM (22). Particularly in the field of T2DM, β-HB can participate in blood glucose regulation and insulin resistance (IR) by influencing the physiological functions of various organ systems including the liver (23), kidney (24) and adipose tissue (25). However, comprehensive reports discussing and summarizing these effects are lacking.

In the present review, a comprehensive search in major databases [PubMed (https://pubmed.ncbi.nlm.nih.gov), Google Scholar (https://scholar.google.com) and Web of Science (https://www.webofscience.com)] up to June 2025 was conducted using the keywords 'β-HB', 'KB', 'β-HB and T2DM', 'KB and T2DM', 'β-HB and T2DM complications' and 'KB and T2DM complications'. Subsequently, the retrieved articles were screened by reading them one by one to exclude irrelevant articles. The present review aimed to clarify the metabolic pathways associated with β-HB, examine its effectiveness and mechanisms of action concerning T2DM and its related complications, to analyze the potential value of endogenous and exogenous ketogenesis methods in increasing β-HB levels as an adjuvant nutritional therapy for T2DM and to provide a reference for subsequent research and clinical translation in this field.

2. Properties of β-HB

Structure of β-HB. β-HB is also known as 3-hydroxybutyric acid or D-3-hydroxybutyrate and has a molecular formula of $C_4H_8O_3$ and a molecular weight of 104 Da. β-HB has two enantiomers: D-β-HB and L-β-HB. In the human body, endogenously produced β-HB in the liver is predominantly in the form of D-β-HB, while L-β-HB is a byproduct generated by certain tissues under ketotic conditions, and its proportion in serum is typically extremely low (26). Compared with D-β-HB, the oxidative metabolism efficiency of L-β-HB is significantly lower. The key enzyme responsible for catalyzing the metabolism of β-HB, namely D-β-hydroxybutyrate dehydrogenase 1 (BDH1), exhibits stereoselectivity; it preferentially catalyzes only the reaction between D-β-HB and acetoacetic acid (ACAC). By contrast, L-β-HB cannot be effectively oxidized by BDH1, resulting in a longer half-life and slower clearance rate in the blood, cells and tissues (27). This notable difference

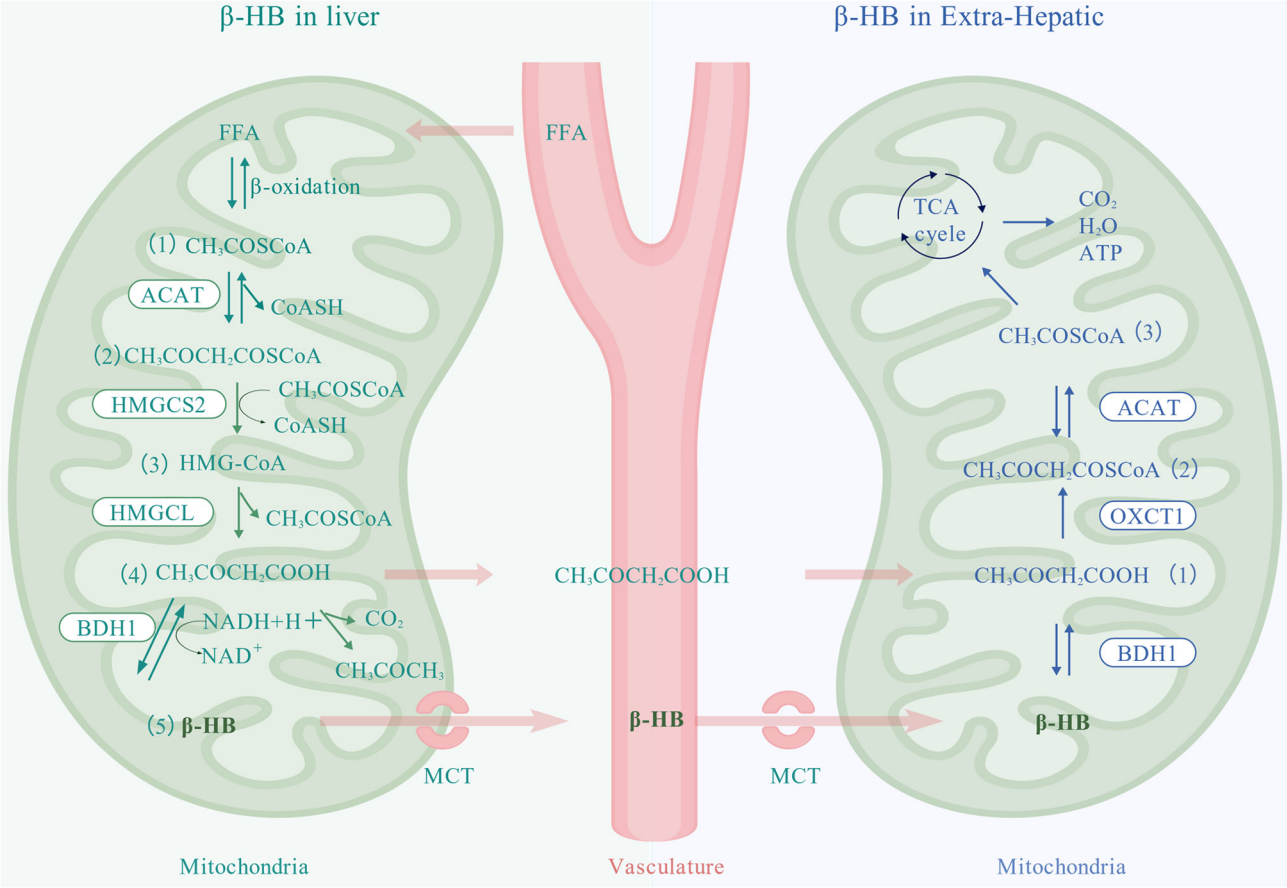

Figure 1. Production of β-HB by the liver and the breakdown of β-HB by extrahepatic tissues. β-HB is synthesized through the following steps: i) The β-oxidation of FFAs results in the production of a substantial amount of acetyl CoA ($CH_3COSCoA$) in the mitochondria of the liver; ii) the condensation of two molecules of acetyl CoA ($CH_3COSCoA$) into acetoacetyl CoA ($CH_3COCH_2COSCoA$) is catalyzed by HMGCS2 with the release of one molecule of CoASH; iii) the condensation of acetoacetyl CoA ($CH_3COCH_2COSCoA$) with another molecule of acetyl CoA ($CH_3COSCoA$) forms HMG-CoA, catalyzed by HMGCL, releasing an additional molecule of CoASH; iv) HMG-CoA is then cleaved by HMG-CoA lyase to produce ACAC (CH_3COCH_2COOH) and acetyl CoA; and v) The reduction of ACAC (CH_3COCH_2COOH) to β-HB is mediated by BDH1, utilizing NADH as the hydrogen donor. A minor fraction of ACAC is converted to acetone (CH_3COCH_3). β-HB is transported by MCTs into the vasculature into the circulatory system and eventually into extrahepatic tissues. The catabolism of β-HB: i) β-HB is dehydrogenated to ACAC (CH_3COCH_2COOH) in the mitochondria of extrahepatic tissues, which is catalyzed by BDH1; ii) ACAC (CH_3COCH_2COOH) is subsequently converted to acetoacetyl CoA ($CH_3COCH_2COSCoA$) by OXCT1; and iii) acetoacetyl CoA (CH3COCH2COSCoA) is catalyzed by ACAT to become acetyl CoA, which then enters the TCA cycle for complete oxidative decomposition to CO_2, H_2O and release of ATP. FFA, free fatty acids; ACAT, acetyl-CoA acetyltransferase; HMGCS2, 3-hydroxymethylglutaryl-CoA synthase 2; HMGCL, 3-hydroxymethylglutaryl-CoA lyase; BDH1, β-hydroxybutyrate dehydrogenase 1; NAD, nicotinamide adenine dinucleotide; β-HB, β-hydroxybutyric acid; TCA, tricarboxylic acid; ATP, adenosine triphosphate; OXCT1, 3-oxoacid CoA transferase 1; MCTs, monocarboxylate transporters; ACAC, acetoacetate; CoA, coenzyme A.

in metabolic utilization efficiency underscores the importance of distinguishing between these enantiomers when considering the signaling functions and therapeutic applications of β-HB.

Metabolic pathway of β-HB

Anabolism of β-HB. β-HB is synthesized from acetyl coenzyme (CoA), which is derived from the β-oxidation of free fatty acids (FFAs). This procedure is facilitated by several enzymes located in the mitochondria of the liver (Fig. 1). The β-oxidation of FFAs yields acetyl CoA, with two acetyl CoA molecules being combined to form acetoacetyl CoA through the action of acetoacetyl CoA sulfatase, releasing one molecule of coenzyme A (CoASH). Afterwards, the enzyme 3-hydroxyglutaryl-CoA (HMG-CoA) synthase catalyzes the reaction between acetoacetyl CoA and an additional acetyl CoA molecule, resulting in the production of HMG-CoA and the release of another CoA molecule. HMG-CoA is then cleaved by 3-hydroxymethylglutaryl-CoA lyase to produce ACAC and acetyl CoA. BDH1 catalyzes the reduction of ACAC to β-HB by using nicotinamide adenine dinucleotide (NADH), while a fraction of ACAC is transformed into acetone. Therefore, ACAC, acetone and β-HB are collectively referred to as KBs (28).

Traditionally, β-HB synthesis is considered to be exclusive to the liver due to the hepatic specificity of the key ketogenesis enzyme, HMG-CoA synthase 2 (19). However, investigations have implicated extrahepatic organization in the production of β-HB and other KBs including glial cells (29,30), kidney (31), pancreatic β-cells (32), retina (33,34) and tumor cells (35). However, the presence of these processes in some of these tissues remains a subject of debate (36).

Catabolism of β-HB. Hepatic tissues contain a robust β-HB synthase system. However, they do not possess an enzyme system for β-HB utilization (14). By contrast, extrahepatic tissues exhibit a well-developed expression of enzymes that utilize β-HB. Consequently, the β-HB that is produced in

the liver is primarily utilized by extrahepatic tissues such as the heart, kidneys, brain and skeletal muscles (14). Within the mitochondria, β-HB is transformed into ACAC and NADH through the action of BDH1. ACAC, along with succinyl CoA, is converted into NADH with the help of succinyl CoA transsulfatase [also termed as 3-oxoacid CoA transferase 1 (OXCT1)], leading to the generation of activated acetoacetyl CoA and succinic acid. The conversion of acetoacetyl CoA is catalyzed by acetyl-CoA acetyltransferase, resulting in the formation of two molecules of acetyl CoA, which subsequently enter the tricarboxylic acid (TCA) cycle for thorough oxidation (Fig. 1) (37). In mammals, glucose, FFAs and KBs (specifically β-HB) are sources of adenosine triphosphate (ATP). Among these sources, β-HB is known to yield the highest amount of ATP per oxygen atom produced (38).

Regulation of β-HB metabolism. Regulation of β-HB metabolism is mainly influenced by factors such as satiety, fasting, carbohydrate metabolism and the activity of specific enzymes. In the presence of satiety or sufficient carbohydrate availability, insulin secretion rises, which leads to a suppression of β-HB production. Conversely, during periods of starvation or when glucose metabolism is impaired, glucagon secretion increases, facilitating the catabolism of β-HB. Malonyl CoA hinders the transport of fatty acyl CoA into the mitochondria by competitively inhibiting carnitine palmitoyltransferase, which leads to a decrease in β-oxidation of fatty acids and a subsequent reduction in β-HB synthesis (28). Additionally, OXCT1 serves as a crucial rate-limiting enzyme in the catabolism of β-HB, and the liver is incapable of metabolizing β-HB due to the absence of OXCT1 (39).

Transport of β-HB. β-HB must be transported across both the plasma membrane and the inner mitochondrial membrane. The primary transporters responsible for β-HB passage at the plasma membrane are the monocarboxylate transporter (MCT) family (40), while at the inner mitochondrial membrane, the pyruvate carrier facilitates its transport (41). There are two types of MCTs: Proton-coupled MCTs and sodium-coupled monocarboxylate transporters (SMCTs). To date, 14 MCTs and 2 SMCTs have been characterized (42-45). Specifically, MCT1, MCT2, MCT4, MCT7 and SMCT1 have been identified as transporters for β-HB (42,44,46-48).

MCT1 is expressed widely in a variety of tissues, such as muscle, kidney, liver and heart (45). MCT2 is expressed in a more limited set of tissues, such as the liver, kidney and testis (49). MCT4 is mainly present in skeletal muscle (50), while MCT7 is detected in the liver, pancreas, skin, vas deferens and testis, as well as other tissues (51). Another significant transporter, SMCT1, is present in the intestine, kidney, thyroid gland and retina (52). This distribution of MCTs and SMCTs suggests a specialized and regulated transport system for β-HB across different tissues.

Functions of β-HB

β-HB as an energetic substrate. Under conditions of intermittent fasting (IF), caloric restriction (CR), KD and exercise, FFA undergoes extensive β-oxidation in the liver, yielding a significant quantity of ACAC. A portion of this ACAC is converted into β-HB (53). β-HB possesses physicochemical properties such as a small molecular weight and good water solubility (54); it can not only be efficiently transported through the blood circulation but also penetrates the blood-brain barrier (BBB) and the capillary walls of muscle tissue, easily reaching extrahepatic tissues where it is oxidized and decomposed as an energy substrate (Fig. 2). Once there, β-HB can be metabolized and utilized, making it a crucial energy source for the brain, heart, kidneys and skeletal muscle, among other tissues, in times of metabolic stress (55). Consequently, β-HB assumes a pivotal role as a compensatory energy fuel during these periods of increased energy demand (56-58). Furthermore, the contribution of β-HB to energy metabolism is notably amplified during the perinatal lactation period and the neonatal phase (59).

β-HB as a signaling molecule. β-HB serves as a mediator for metabolic signaling that influences numerous cellular processes (Fig. 2). β-HB is a ligand for the G protein-coupled receptors (GPCRs), GPR109A (also known as HM74A in humans and PUMA-G in mice) and GPR41. GPR109A is found in adipocytes, retinal tissue and macrophages. Within physiologically relevant concentrations (Ki=0.7 mM), β-HB selectively stimulates GPR109A, leading to the activation or inhibition of various signaling pathways linked to lipid metabolism and cellular growth (60). Moreover, GPR41, also known as free fatty acid receptor 3, is present in sympathetic ganglia; it can inhibit sympathetic activity in mice through the G protein β-γ complex/phospholipase C β/MAPK signaling pathway, thereby suppressing the overall metabolic rate (61,62). Besides GPCRs, β-HB engages directly with ribonucleoproteins; it plays a role in histone acetylation (63), histone lysine β-hydroxybutyrylation (Kbhb) (64) and indirectly promotes protein hyperacetylation (65). Furthermore, β-HB exerts direct regulatory influences on K+ channels and neuronal vesicular glutamate transporters (66) and it inhibits inflammation mediated by the NOD-like receptor family pyrin domain-containing 3 (NLRP3) (67).

3. β-HB and ketosis

Under normal circumstances, the concentration of β-HB in human plasma and tissues is maintained at ~0.1 mM (68). Under conditions of prolonged fasting or a KD, insufficient carbohydrate intake prompts the body to reduce protein breakdown to maintain blood glucose levels. Instead, it shifts to fat breakdown to produce KBs, which serve as an alternative energy source to glucose. During this period, the KB levels of the body increase slightly (69). Pathological states such as obesity and DM, when the KB production of the body exceeds its utilization capacity and accumulates, lead to ketoacidosis (KA). Ketosis is characterized by elevated serum KB levels and is classified into nutritional ketosis (NK) and pathological KA. The core differences between the two lie in KB concentration, acid-base balance status and inducing mechanisms (58,70). As a key hormone regulating ketogenesis, insulin maintains KB homeostasis primarily through three mechanisms: i) It inhibits lipolysis in adipose tissue, reducing the transport of FFAs to the liver; ii) it directly decreases the activity of enzymes involved in KB synthesis in the liver; and iii) it enhances the efficiency of KB oxidation and utilization in peripheral tissues such as the brain and muscles (71).

NK. NK is an adaptive metabolic state in the body where KBs serve as the primary energy source under specific

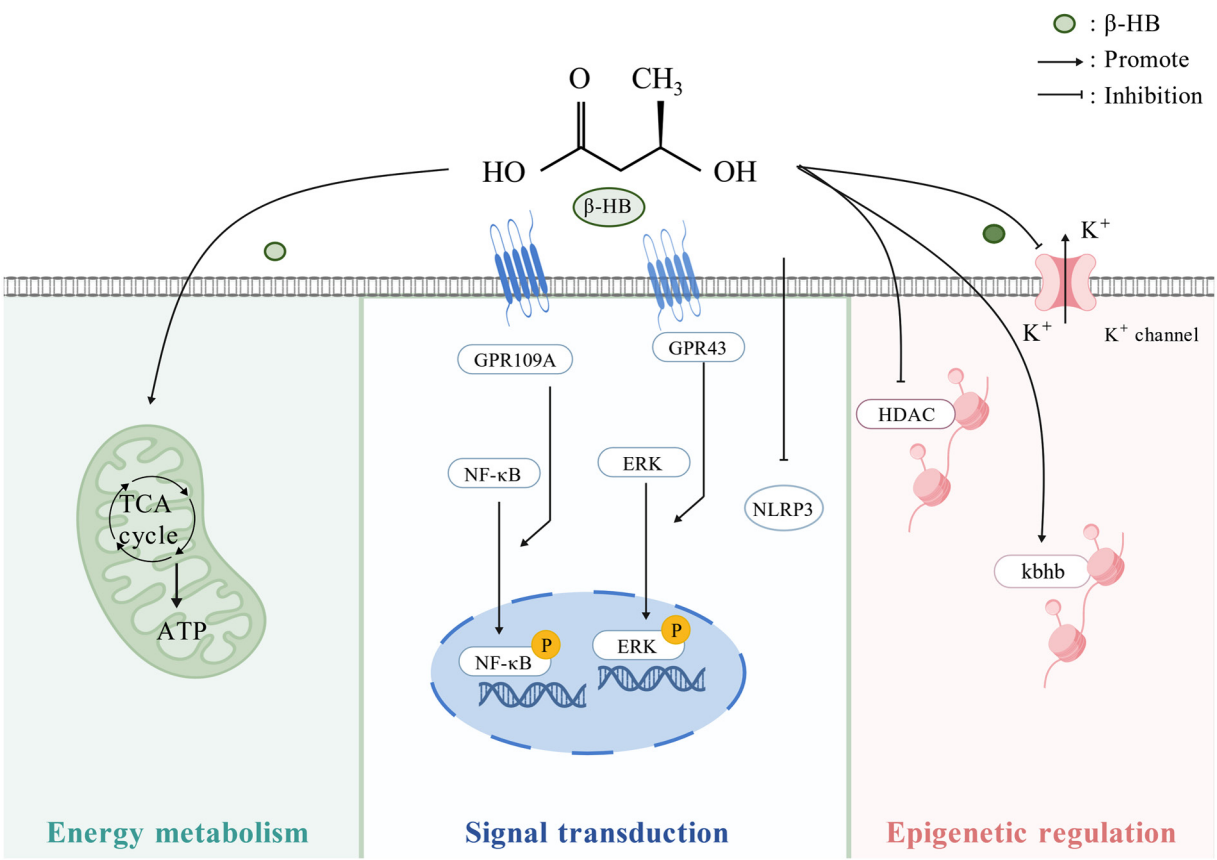

Figure 2. Biological functions of β-HB. β-HB acts as an energy substrate to regulate metabolic reactions as a signaling molecule that binds to the ligand of the GPCR to modulate downstream signaling molecules and inhibits NLRP3. As an epigenetic regulator, β-HB inhibits HDAC, promotes Kbhb and controls K^+ channels. HDAC, histone deacetylase; Kbhb, lysine β-hydroxybutyrylation; GPCRs, G protein-coupled receptors; NF-κB: nuclear factor κB; ERK, extracellular regulated protein kinase; TCA, tricarboxylic acid; ATP, adenosine triphosphate; NLRP3, NOD-like receptor family pyrin domain containing 3; β-HB, β-hydroxybutyric acid.

physiological conditions or dietary interventions. NK is typically induced by factors such as starvation, IF, KD or prolonged exercise. The criterion widely accepted in most studies for diagnosing NK is a serum β-HB level ranging from 0.5 to 3 mmol/l (72). From the perspective of metabolic effects, NK has clear physiological advantages, such as improving insulin sensitivity and optimizing energy utilization efficiency (73-76). Moreover, although the blood glucose level of the body decreases slightly in this state, the blood pH value remains within the normal range at all times. It should be noted that the induction process of NK may be accompanied by transient discomfort symptoms such as drowsiness and dizziness. Additionally, long-term dietary interventions (such as strict KD) may have potential impacts on the homeostasis of intestinal flora. Meanwhile, due to the high requirements for dietary adherence, most individuals find it difficult to persist with such interventions over the long term (77). In the ketogenic state, on one hand, the low-insulin environment allows the liver to activate the ketogenesis pathway to supplement energy; on the other hand, insulin can increase the concentration of malonyl-CoA by activating acetyl-CoA carboxylase, thereby inhibiting the activity of carnitine palmitoyl transferase 1 (CPT-1), restricting the excessive entry of fatty acids into the mitochondria, and ultimately precisely controlling the blood ketone concentration within the safe range of NK (78).

Diabetic ketoacidosis (DKA). DKA is the most common acute hyperglycemic emergency in patients with DM. Among these patients, those with T1DM are at a high risk of developing DKA due to absolute insulin deficiency (58). The typical clinical features of DKA are characterized by a triad of hyperglycemia (blood glucose ≥13.9 mmol/l), hyperketonemia (typically β-HB of ≥3.0 mM) and hyperketonuria (urinary KB test strip ≥2+), along with electrolyte disturbances and acid-base imbalance (79,80). Due to the absolute insulin deficiency, the inhibition of hormone-sensitive lipase is lifted, leading to a surge in FFAs; a sharp drop in malonyl-CoA causes excessive activation of CPT-1 and, combined with the upregulation of ketogenic enzymes, this results in an abnormal increase in KB production. In the state of DKA, the level of β-HB can rise to 10-20 mM (or even higher) (81).

4. β-HB in T2DM and its complications

T2DM arises from the interplay of two main factors: Pancreatic β-cells exhibiting impaired insulin secretion and tissues sensitive to insulin exhibiting an inadequate response. Inflammation, endoplasmic reticulum stress (ERS) and metabolic/oxidative stress have been identified as potential contributors to β cell dysfunction or IR (82-84). Research has underscored the role of β-HB in antioxidant, anti-inflammatory and mitochondrial function-protective mechanisms (85). It is suggested that

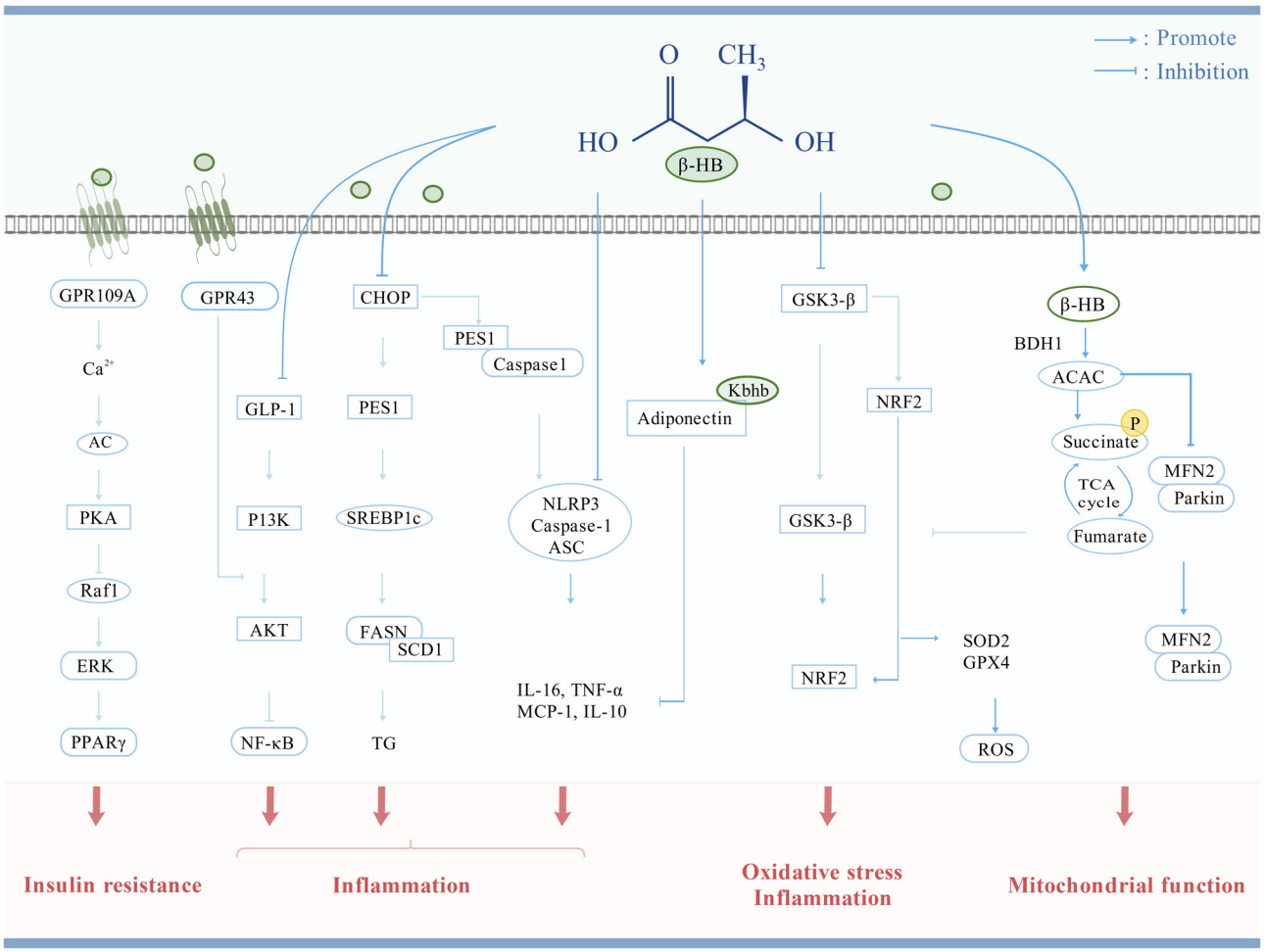

Figure 3. Possible mechanisms of β-HB against T2DM. β-HB acts as a signaling molecule to regulate IR, inflammation, oxidative stress, mitochondrial function and cell apoptosis to improve T2DM. GPR, G protein-coupled receptor; AC, adenylyl cyclase; PKA, protein kinase A; Raf1, Raf-1 proto-oncogene, serine/threonine-protein kinase; ERK, extracellular regulated protein kinase; PPARγ, peroxisome proliferator-activated receptor γ; GLP-1, glucagon-like peptide-1; P13K, phosphatidylinositol-3-kinase; NF-κB: nuclear factor-κB; CHOP, C/EBP-homologous protein; PES1, pescadillo 1; SREBP1c, sterol regulatory element binding protein 1c; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; TG, triglyceride; Caspase-1, cysteinyl aspartate specific proteinase 1; NLRP3, NOD-like receptor family pyrin domain-containing 3; TNF-α, tumor necrosis factor-α; MCP1, monocyte chemoattractant protein 1; BDH1, β-hydroxybutyrate dehydrogenase 1; ACAC, acetoacetate; TCA, tricarboxylic acid; MFN2, mitofusin 2; Nrf2, nuclear factor-erythroid 2-related factor 2; GSK3-β, glycogen synthase kinase 3-β; SOD, superoxide dismutase; GPX4, glutathione peroxidase 4; ROS, reactive oxygen species; β-hydroxybutyric acid; T2DM, type 2 diabetes mellitus.

β-HB may regulate the occurrence and development of T2DM through these mechanisms. This provides a new clinical diagnosis and treatment avenue for the early diagnosis and management of various complications of T2DM; however, its underlying mechanism of action still needs further exploration and clarification (Fig. 3 and Table I).

Potential effects of β-HB on the liver in T2DM

Effects of T2DM on the liver. The liver is essential for regulating glucose and lipid metabolism and it is a key site for the development of IR. Chronic increase in plasma-free FFA leads to metabolic imbalance in the body and induces IR, which promotes FFA delivery to the liver and hepatic fat deposition. Increased hepatic fat accumulation results in heightened lipotoxicity, adipose tissue inflammation, impaired mitochondrial function and ERS. Excessive deposition of hepatic extracellular matrix forms hepatic fibrosis, which ultimately leads to cirrhosis; T2DM hepatic fibrosis is one of its pathological manifestations (86). It is noteworthy that the abnormal activation of the hepatic gluconeogenesis pathway is a crucial mechanism leading to the hyperglycemic state. This process is regulated by key gluconeogenic enzymes and the increased activity of these enzymes significantly promotes gluconeogenesis, thereby exacerbating hyperglycemia (87).

Effects of β-HB on the liver. A persistent, low-grade inflammatory state is recognized as a pivotal element of IR and metabolic disorders. Zhou *et al* (23) demonstrated that β-HB can inhibit lipid synthesis in the hepatocytes of KKAy mice (T2DM mouse model), thereby reducing hepatic lipid accumulation and decreasing the expression of hepatic inflammatory factors. The mechanism may involve β-HB impairing the binding ability of the transcription factor, C/EBP homologous protein, to the promoters of ribosome biogenesis factor 1 (PES1), thereby leading to downregulated expression of the PES1 protein. Ultimately, this improves liver pathology through two key effects: i) It inhibits the binding of PES1 to the promoters of E1A-binding protein p300 (p300) and cysteine-aspartic acid protease 1; and ii) it reduces p300-mediated acetylation of

Table I. Role of β-HB in T2DM and its complications and related mechanisms.

A, Liver						
Authors, year	*In vivo/ in vitro*	Model	Methods for intervening in β-HB	Main findings	Mechanisms and indicators of change	(Refs.)
Zhou *et al*, 2022	*In vivo*	KKAy mice	KD	B-HB reduced the hepatic adipose tissue inflammatory response and improved hepatic lipid metabolism in KKAy mice.	CHOP, PES1, p300, SREBP1c, ↓: N'-SREBP1c, FASN, SCD1, NLRP3, Caspase1, cleaved-Caspase1, IL-1β, IL-18	(23)
B, Kidney						
Guo *et al*, 2023	*In vivo*	HFD feeding combined with STZ injected to induce T2DM mice	1,3-BDO solution as a drinking solution.	Supplementation with 1,3-BDO reprograms energy metabolism and attenuates kidney damage.	↑: ATP, β-HB	(24)
Wan *et al*, 2023	*In vivo*	db/db mice	Supplementation of β-HB at 100 mM in drinking water for 6 weeks.	B-HB prevents diabetic environmentally-induced glomerular podocyte senescence and injury.	↑: BDH1, Nrf2	(95)
Wan *et al*, 2023	*In vitro*	HK-2 cells	5 mM treatment for 48 h.	B-HB prevents diabetic environmentally-induced glomerular podocyte senescence and injury.	↓: ROS, IL-1β, IL-18; ↑: Nrf2, ACAC, succinate, fumarate	(95)
Fang *et al*, 2021	*In vivo*	STZ injected	Intraperitoneal injection of β-HB (100 mg/kg/day) every other day for 4 weeks	B-HB reduces albuminuria, renal hypertrophy and histologic signs of DKD	↓: Urinary protein, p-GSK3β; ↑: Nrf2, GSK3β	(96)
Fang *et al*, 2021	*In vivo*	Glomerular podocytes treated with PA combined with TGF-β	4 mM treatment for 48 h	B-HB enhances the antioxidant response and ultimately attenuates podocyte senescence.	↓: p-GSK3β; ↑: Nrf2, GSK3β	(96)

Table I. Continued.

C, Adipose

Authors, year	*In vivo*/ *in vitro*	Model	Methods for intervening in β-HB	Main findings	Mechanisms and indicators of change	(Refs.)
Zhang *et al*, 2023	*In vivo*	HFD feeding combined with STZ injected to induce T2DM mice.	10% 1,3-BDO solution as a drinking solution.	B-HB reduces fasting blood glucose levels and improves glucose tolerance and IR in T2DM mice via HCAR2.	↓: Raf-1, ERK1/2, p-PPARγ; ↑: PPARγ, Ca^{2+}, AC, cAMP, PKA.	(22)
Park *et al*, 2011	*In vivo*	Rats had a 90% Px.	150 mg/kg of β-HB was injected intraperitoneally twice daily into rats for 5 weeks.	Intraperitoneal injection of β-HB decreased epididymal fat pads and serum leptin levels.	↓: PEPCK; ↑: IRS2, p-AKT/AKT	(107)

D, Blood vessel

Wang *et al*, 2023	*In vivo*	db/db mice	KD (5% carbohydrate, 75% fat and 20% protein).	KD inhibits the T2DM-induced increase in PES1, which may lead to vascular hyperpermeability in T2DM mice through the ubiquitination of VE-cadherin.	↓: PES1, VEGF; ↑: VE-cadherin, Occludin	(116)
Wang *et al*, 2023	*In vitro*	MVECs intervened with HG.	2 mM β-HB intervention for 24 h.	β-HB reduces the ubiquitination of VE-cadherin promoted by PES1.	↓: PES1, VEGF; ↑:VE-cadherin, Occludin	(116)

E, Heart

Thai *et al*, 2021	*In vivo*	db/db mice	KE feeding 4 weeks.	KE supplementation prevents progression toward DCM in T2DM by limiting oxidative stress and enhancing mitochondrial quality control via mitophagy.	↓: H_2O_2; ↑: BDH1, OXCT1, ACAT, mitochondrial complex II, mitochondrial complex IV, mitochondrial complex V, GPX4, Parkin, Mfn2, pro-LC3B.	(117)

Table I. Continued.

Authors, year	*In vivo*/ *in vitro*	Model	Methods for intervening in β-HB	Main findings	Mechanisms and indicators of change	(Refs.)
F, Brain						
Park *et al*, 2011	*In vivo*	Px diabetic rats	B-HB injection at 12 mg/h for 28 days.	β-HB central infusion improves hypothalamic leptin and insulin signaling.	↑: IRS2, p-AKT, GLUT2, glucokinase, STAT3	(130)
G, Retina						
Trotta *et al*, 2019	*In vivo*	STZ-injected mice	Inject twice weekly for 10 weeks at 25, 50 and 100 mg/kg.	β-HB attenuates retinal NLRP3 inflammatory vesicle activation markers, reduces apoptotic cells and improves retinal permeability and homeostasis.	↓: NLRP3, ASC, caspase–1, IL–1β, IL–18, p-PERK, p-IRE1, ATF6α; ↑: Connexin 43, HCA2	(141)

↑, increase; ↓, decrease; KD, ketogenic diet; β-HB, β-hydroxybutyric acid; CHOP, C/EBP-homologous protein; PES1, promoter of ribosomal biogenesis factor 1; SREBP1c, sterol regulatory element binding protein 1c; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; NLRP3, NOD-like receptor (NLR) family pyrin domain-containing 3; Caspase1, cysteinyl aspartate specific proteinase 1; HFD, High-fat diet; STZ, streptozotocin; T2DM, Type 2 diabetes mellitus; 1,3-BDO, 1,3-butanediol; ATP, adenosine triphosphate; BDH1, β-hydroxybutyrate dehydrogenase 1; ROS, reactive oxygen species; Nrf2, nuclear factor-erythroid 2-related factor 2; ACAC, acetoacetic acid; TGF-β, transforming growth factor-β; GSK3β, glycogen synthase kinase 3β; Raf-1, Raf-1 proto-oncogene, serine/threonine-protein kinase; ERK1/2, extracellular regulated protein kinases 1/2; PPARγ, peroxisome proliferator-activated receptor γ; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; IRS2, insulin receptor substrate; AKT, protein kinase B; PES1, pescadillo ribosomal biogenesis factor 1; VE-cadherin, vascular endothelial cadherin; VEGF, vascular endothelial growth factor; KE, ketone esters; DCM, diabetic cardiomyopathy; OXCT1, 3-oxoacid CoA transferase 1; ACAT, acetyl-CoA acetyltransferase; GPX4, glutathione peroxidase 4; Mfn2, mitofusin 2; LC3B, microtubule-associated protein 1 light chain 3; Px, pancreatectomy; GLUT2, glucose transporter protein 2; STAT3, signal transducer and activator of transcription 3B; ASC, apoptosis-associated speck-like protein containing a CARD; p-EPCK, phosphoenol-pyruvate carboxykinase; p-IRE1, phosphorylated inositol-requiring enzyme 1; ATF6α, activating transcription factor 6α; HCA2, hydroxycarboxylic acid receptor 2.

sterol regulatory element-binding protein 1c (SREBP1c) and the associated inflammatory response pathways. SREBP1c is a core transcription factor for lipid synthesis, and the inhibition of its activity can directly reduce hepatic triglyceride synthesis. Furthermore, the authors observed that KD increased the levels of circulating β-HB, suppressed hepatic PES1 expression and improved hepatic lipid regulation, the inflammatory response, blood glucose levels and IR in KKAy mice (23).

The accumulation of reactive oxygen species (ROS) caused by excessive hepatic lipid deposition is a crucial driving factor for hepatic fibrosis in T2DM (88). Xu *et al* (89) discovered that upregulation of BDH1 reduces fibrosis, inflammation and apoptosis in the livers of db/db mice, processes that are mediated by ROS. The underlying mechanism may be that β-HB metabolism mediated by BDH1 upregulates the production of fumarate. In turn, fumarate can inhibit the activity of Kelch-like ECH-associated protein 1 (Keap1), thereby activating nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is a core transcription factor for resisting oxidative stress; its activation can induce the expression of antioxidant enzymes, which in turn scavenge excessive ROS (89). BDH1 promotes the interconversion of β-HB with ACAC (37). Therefore, BDH1-mediated hepatic β-HB metabolism opens a new avenue for the treatment of db/db mice.

Effects of β-HB on the kidneys in T2DM

Effects of T2DM on the kidneys. The kidneys are one of the most susceptible target organs in T2DM. Diabetic kidney disease (DKD) caused by T2DM has become the leading cause of end-stage renal disease and ~40% of T2DM patients will progress to DKD (90). Persistent hyperglycemia can induce functional abnormalities in the glomerular feedback system and mediate cellular damage via glucotoxicity, thereby triggering a state of glomerular hyperfiltration. This early pathological change can further activate a series of cascade reactions, including metabolic disorders, hemodynamic abnormalities, ERS, inflammatory responses and fibrotic processes, leading DKD to progress from early functional impairment to irreversible organic lesions (91). Patients with DKD frequently exhibit early signs of hyperfiltration and albuminuria, as well as glomerular and tubular lesions (92).

Effects of β-HB on the kidneys. SMCT1, as a high-affinity transporter for monocarboxylates, is a key molecule for β-HB uptake in tissues (93). Under the state of hyperinsulinemia in T2DM, the protective effect of β-HB on renal tubular epithelial cells may depend on the 'β-HB uptake and utilization' mediated by SMCT1. The authors (24) observed a decrease in renal SMCT1 expression in patients with DKD and in mice with T2DM. Following overexpression of SMCT1, the levels of β-HB in the serum and kidneys of T2DM mice increased, while the urinary β-HB levels decreased and renal energy metabolism improved. By contrast, a lack of the SLc5α8 gene responsible for encoding SMCT1 led to structural damage and functional impairment of the renal tubules in T2DM. However dietary supplementation with 1,3-butanediol (1,3-BDO), a precursor of β-HB, can ameliorate renal injury in SMCT1-knockout mice (24). Mechanistic studies have shown that hyperinsulinemia inhibits SMCT1, impairs the uptake of β-HB and thereby compromises mitochondrial function and cell survival in renal tubular epithelial cells (94). Therefore, the transport of β-HB into renal tissues mediated by SMCT1 is a necessary process for maintaining the mitochondrial function of renal tubules. In addition, renal β-HB metabolism mediated by BDH1 may also provide a new therapeutic approach for DKD. Both KD intervention and β-HB intervention can increase the serum β-HB levels and upregulate the renal expression of BDH1, thereby ameliorating DKD (95). Mechanistically, β-HB metabolism mediated by BDH1 upregulates the production of fumarate, which in turn activates Nrf2 to inhibit oxidative stress and alleviate renal tubular injury (95). The protective effect of β-HB on the kidney is not limited to renal tubules; it also exerts a protective effect on glomerular cells. Supplementation with β-HB can reduce albuminuria and alleviate renal hypertrophy in mice with DKD (96). *In vitro* experiments have confirmed that β-HB can protect glomerular podocytes from injury and senescence under stimulation by high glucose combined with transforming growth factor-β (TGF-β). The underlying mechanism may be as follows: β-HB inhibits the activity of glycogen synthase kinase 3β and the phosphorylation of Nrf2, reduces the nuclear export of Nrf2 and increases its nuclear accumulation, thereby enhancing the antioxidant response and delaying podocyte senescence (96). In summary, β-HB protects renal tubules and glomeruli in DKD from oxidative stress-induced injury by inhibiting the nuclear translocation of Nrf2.

Effect of β-HB on adipose tissue in T2DM

Effects of T2DM on adipose tissue. It has been indicated that ~80% of individuals with T2DM exhibit signs of overweight or obesity (97). When adipose tissue exceeds its normal storage capacity, it accumulates ectopically (such as in the heart, skeletal muscle, liver and pancreas), leading to increased visceral lipid deposition and the onset of FFA-induced toxicity, commonly referred to as lipotoxicity (98). Lipotoxicity denotes the harmful effects of lipid byproducts on tissues that are not composed of fat, such as the liver, skeletal muscle, heart, kidneys and pancreatic β-cells (99), which are pivotal in the pathogenesis of T2DM IR (100). Furthermore, under healthy conditions, adipokines (such as adiponectin) secreted by adipose tissue can enhance insulin sensitivity. By contrast, the 'endocrine dysfunction' of adipose tissue under pathological states exhibits pathogenicity (101). The increase in adipose tissue mass leads to low-grade inflammation by altering the secretion of adipokines and cytokines, and the production of these adipocytokines is negatively correlated with IR in T2DM (102).

Effects of β-HB on adipose tissue. Zhang *et al* (22) employed a db/db mouse model alongside a T2DM mouse model induced by a high-fat diet (HFD) and treated with streptozocin to investigate how administering a 10% aqueous solution of 1,3-BDO affected these mice. It was discovered that β-HB binds to hydroxycarboxylic acid receptor 2 (HCAR2) and triggers an increase in intracellular Ca^{2+} levels within adipocytes. This process triggers adenylate cyclase, leading to an increase in cyclic adenosine monophosphate (cAMP) levels, which in turn activates protein kinase A (PKA). When activated, PKA restrains the activity of Raf-1 proto-oncogene serine/threonine-protein kinase (Raf1), causing a decrease in the activity of extracellular regulated protein kinases 1/2 (ERK1/2). This reduction ultimately inhibits the phosphorylation of

peroxisome proliferator-activated receptor γ (PPARγ) at Ser273 in adipocytes. Such changes in the expression levels of genes regulated by PPARγ contribute to a decrease in IR (22). As a result, β-HB regulates the activity of ERK1/2 through the HCAR2/Ca^{+}/cAMP/PKA/Raf1 pathway, ultimately enhancing PPARγ function through post-translational changes and decreasing IR linked to T2DM.

Insufficient secretion of adiponectin is a core feature of adipose tissue dysfunction in T2DM. Nishitani *et al* (25) found that in KKAy mice, the serum level of β-HB was increased, while in periovarian adipose tissue (visceral adipose tissue), the expression of adiponectin was decreased, the inflammatory response was enhanced, the metabolism of the insulin signaling pathway was weakened, the expression levels of MCT1 and MCT2 were downregulated and the expression of OXCT1 (the enzyme responsible for KB catabolism) was reduced. These findings suggest that abnormal β-HB metabolism is closely associated with adipose tissue dysfunction. In *in vitro* intervention experiments on 3T3-L1 adipocytes, β-HB can significantly upregulate the mRNA and protein expression levels of adiponectin, while inhibiting inflammatory responses (25). Further mechanistic studies have shown that the regulatory effect of β-HB depends on a novel epigenetic modification, Kbhb. Specifically, β-HB can induce an increase in the Kbhb level of the histone H3 lysine 9 (H3K9) site within the adiponectin gene promoter region in 3T3-L1 adipocytes. This modification serves to open the chromatin structure of the adiponectin gene, facilitate the binding of transcription factors, and thereby activating the expression of the adiponectin gene. Lipocalin expression has also been found to be regulated by two epigenetic modifications (103,104) and promoter activity (105,106), and lipocalin gene expression has been reported to be epigenetically associated with 3T3-L1 adipocytes (104). The research findings of Nishitani *et al* (25) revealed that β-HB induces an increase in Kbhb at the H3K9 site in 3T3-L1 adipocytes. Therefore, β-HB seems to offer defense in KKAy mice by inducing epigenetic changes in the lipocalin gene within adipocytes. β-HB can directly regulate adiponectin gene expression through epigenetic modifications and this mechanism provides a new perspective for adipose tissue protection.

The positive effects of KD on managing energy metabolism and maintaining glucose homeostasis are still a topic of contention. A notable detail is that KD and the intraperitoneal administration of β-HB produce distinct effects on lipid metabolism. Park *et al* (107) observed that KD led to an increase in epididymal fat pads and serum leptin levels, while leptin-related signal transducer and activator of transcription 3 (STAT3) signaling was impaired, leading to visceral fat accumulation in diabetic rats with 90% pancreatectomy (Px). By contrast, intraperitoneal administration of β-HB led to decreased testicular fat pads and serum leptin levels, partially restoring hepatic insulin receptor expression and signaling without affecting STAT3 signaling. This discrepancy may be attributed to the fact that a KD inhibits hypothalamic STAT3 phosphorylation, thereby inducing leptin resistance, whereas β-HB injection exerts no effect on STAT3 signal transduction. Additionally, the high-fat content in a KD leads to an increase in FFAs, which promotes fat accumulation. Apart from β-HB, a KD contains substantial amounts of saturated fats and trans fats; these components themselves can disrupt lipid metabolism (such as increasing FFA levels and inducing inflammation) (108). These findings suggest that β-HB exerts a protective effect on lipid metabolism, while a KD is not suitable for lipid metabolism management in non-obese patients with T2DM. Additionally, research has shown that the supraphysiological levels of β-HB (ranging from 15 to 50 mM) triggers the occurrence of lipofuscinosis (109), while it does not promote lipo-browning at physiological concentrations (110).

Effects of β-HB on the cardiovascular system in T2DM

Effects of T2DM on the cardiovascular system. T2DM is strongly correlated with the development of cardiovascular disease (111). The metabolic environment characterized by hyperglycemia and hyperlipidemia in patients with T2DM increases the risk of developing heart disease (112). Diabetic cardiomyopathy (DCM) refers to heart diseases complicated by or associated with DM, including coronary atherosclerotic heart disease, DCM itself and arrhythmias and cardiac dysfunction caused by autonomic nerve disorders (113). The main pathological features of DCM include cardiomyocyte hypertrophy, myocardial fibrosis and impaired coronary microvascular perfusion. The pathogenesis involves oxidative stress, inflammation and impaired Ca^{2+} handling as well as alterations in substrate metabolism/utilization, insulin signaling, gene regulation, mitochondrial dysfunction, ERS, neurohumoral activation and cardiomyocyte death (114). However, there is currently a lack of effective therapeutic approaches for DCM (115).

Effects of β-HB on the cardiovascular system. β-HB, as a key metabolite of a KD, downregulates the expression of vascular PES1, thereby inhibiting PES1-mediated ubiquitin-dependent degradation of vascular endothelial cadherin (VE-cadherin). Consequently, β-HB upregulates barrier-protective proteins such as VE-cadherin and downregulates pro-leakage proteins such as vascular endothelial growth factor (VEGF), ultimately improving vascular hyperpermeability in T2DM mice; it also assists in reducing fasting blood glucose (116). This finding provides a novel 'PES1/β-HB-targeted' direction for the treatment of vascular complications in T2DM. Ketone esters (KEs) increase the circulating level of β-HB and upregulate the expression of myocardial BDH1, ultimately improving cardiac function in mice with T2DM. The underlying mechanism may be associated with β-HB regulating the expression of mitofusin 2, promoting Parkin-mediated mitophagy and enhancing mitochondrial biogenesis. These processes collectively optimize mitochondrial quality control and reduce the level of oxidative stress (117). Another study also indirectly demonstrated the myocardial antioxidant stress effect of β-HB. Lin *et al* (118) observed that the expression of BDH1 was decreased in the aorta of T2DM model mice, and systemic overexpression of BDH1 reduced the area of atherosclerotic plaques in T2DM. In their *in vitro* studies, BDH1 was found to mitigate oxidative stress and inflammatory reactions in Raw264.7 cells (mouse macrophage cell line) by enhancing ferredoxin metabolic flux and stimulating the Nrf2 signaling pathway. The study by Uchihashi *et al* (119) further supports their conclusion. This study showed that heart-specific overexpression of BDH1 can also improve oxidative stress and cardiac remodeling in heart

failure induced by pressure overload. In addition, the reduction in β-HB concentration can be considered a marker of overall FFA oxidation (120). Liepinsh *et al* (121) found that the plasma β-HB level in Goto-Kakizaki (GK) rats was significantly decreased, while after treatment with light phosphate at a dose of 200 mg/kg for 4 and 8 weeks, the β-HB concentration was further reduced. Therefore, the authors concluded that the cardioprotective effect of light phosphate treatment in GK rats could be explained by partial inhibition of FFA oxidation and increased glucose metabolism.

Effects of β-HB on the brain in T2DM

Effects of T2DM on the central nervous system. Individuals with T2DM exhibit an accelerated rate of brain aging at ~26% faster than those without diabetes (122). The effect of diabetes on the central nervous system has garnered considerable attention in recent years. 'Diabetic encephalopathy (DE)' was raised by Reske-Nielsen *et al* (123) to describe a central nervous system complication associated with diabetes, characterized by cognitive and behavioral deficits. Clinically, DE presents as cognitive dysfunction, decision-making disorders and mood disorders, and pathologically as structural and functional alterations in intracranial tissues. The pathological hallmarks of DE include gray matter, white matter, hippocampal atrophy, compromised synaptic plasticity, glial cell dysfunction and alterations in the structure and function of cerebral blood vessels. The pathological mechanisms of DE encompass an imbalance in pancreatic amyloid polypeptide homeostasis, microRNAs, macrophage autophagy, Lipin1, advanced glycation end products (AGEs), oxidative stress, hyperphosphorylation of Tau proteins and intestinal homeostasis dysregulation (124,125).

Effects of β-HB on the central nervous system in T2DM. β-HB is produced in the liver and it can traverse the BBB to provide energy to the brain when glucose levels are low (126). Andersen *et al* (127) found that cerebral glucose metabolism was reduced in db/db mice, whereas hippocampal β-HB metabolism was increased. Furthermore, an enhancement in mitochondrial oxygen consumption and the rate of ATP synthesis was observed. This suggests that β-HB can partially compensate for insufficient glucose metabolism by enhancing mitochondrial oxidation, thereby maintaining the energy homeostasis of hippocampal neurons. The entry of β-HB into nerve cells depends on MCTs. Pierre *et al* (128) reported that, at 6 weeks of age, the hippocampus of db/db mice exhibit increased levels of MCT1 and MCT2, and that this upregulated expression of these transporter proteins may support the utilization of KBs. MCT1 is expressed by endothelial cells, astrocytes, oligodendrocytes and microglial cells in the brain, whereas MCT2 is primarily expressed by neurons (129). Thus, the augmented KB metabolism in db/db mice may be related to the expression levels of neuronal transporter proteins.

The hypothalamus, a pivotal regulator of energy homeostasis, has been a subject of debate in terms of its potential influence on energy and glucose homeostasis through central KBs. Park *et al* (130) administered β-HB at a dose of 12 μg/h into the lateral ventricle of diabetic rats with 90% Px. After 28 days, they observed increased β-HB levels in the hypothalamus and liver, enhanced leptin and insulin signaling in the hypothalamus and elevated STAT3 phosphorylation. By contrast, intraperitoneal injection of β-HB has no effect on hypothalamic signaling in Px rats (107). This discrepancy may be caused by the BBB: β-HB administered via intraperitoneal injection needs to cross the BBB through MCTs. At the cellular and molecular levels, β-HB exhibits multifaceted neuroprotective potential. Majrashi *et al* (131) demonstrated that supplementation of β-HB at doses of 250 and 500 μM exert a neuroprotective effect on HT22 cells (mouse hippocampal neuronal cell line). The proliferative effect of hippocampal neurons can reduce oxidative stress, maintain energy metabolism, improve mitochondrial function and regulate cell apoptosis. Combined with computational pharmacokinetic and molecular modeling analyses. This study further confirmed the neuroprotective potential of β-HB in cognition-related neurodegenerative diseases. The brain is one of the organs with the highest lipid content and lipids account for ~50% of its dry weight. Dabke *et al* (132) simulated the effect of endogenous β-HB production induced by a KD on the lipids of neuronal cells. Their research showed that when HT22 cells incubated under low-glucose conditions were treated with β-HB at 5 mM, the levels of cholesterol and phosphatidylserine decreased, while the ratio of phospholipids to cholesterol increased.

Effects of β-HB on the retina in T2DM

Effects of T2DM on the retina. DM is linked to various eye-related issues, such as diabetic retinopathy (DR), cataracts, diabetic papillopathy, glaucoma and diseases affecting the ocular surface (133). DR is a major complication of DM that manifests as retinal microangiopathy and stands as the leading cause of vision impairment in middle-aged individuals (134). The condition of diabetes enhances the permeability of the blood-retinal barrier, promoting angiogenesis in the retina (135). The development of DR is complex, and it involves increased production of free radicals, the stimulation of AMP-activated protein kinase/mammalian target of rapamycin signaling pathways, the activation of the renin-angiotensin system, engagement of TGF-β/Smad signaling, the kinin system involving kinin-releasing enzymes, the accumulation of AGEs and various inflammatory agents such as VEGF (136-138). The abnormal signaling pathway of TGF-β plays a role in the development of DR. Systemic inhibition of TGF-β signaling offers protection against obesity, diabetes and liver fat accumulation in mice (139). However, TGF-β has been proposed to safeguard retinal ganglion cells against oxidative harm induced by hyperglycemia by enhancing cellular antioxidant and neuroprotective mechanisms, such as the Nrf2/Keap1 pathway (140).

Effects of β-HB on the retina in T2DM. Trotta *et al* (141) demonstrated that diabetic mice with heightened ERS markers [phosphorylated (p)ERK, phosphorylated inositol requiring enzyme 1 and activating transcription factor 6α], increased NLRP3 inflammasome activity (NLRP3, apoptosis associated speck-like protein containing a CARD and caspase-1) and increased levels of pro-inflammatory cytokines (IL-1β and IL-18) experienced significant reductions in these parameters following intraperitoneal injections of 50 and 100 mg/kg β-HB. The injections led to increased plasma and retinal β-HB levels as well as enhanced expression of the β-HB receptor, GPR109A. Consequently, ERS markers, NLRP3 inflammasome

activation markers and pro-inflammatory cytokine levels were significantly reduced. Moreover, retinal outer nuclear layer cell death was diminished, effectively safeguarding the retina from diabetic-induced damage. Therefore, β-HB may offer protection to the retinas of diabetic mice by mitigating inflammation and ERS through the GPR109A receptor.

5. Application of β-HB in the clinical setting of T2DM

Clinical research on β-HB mainly includes three aspects: i) The potential of β-HB as a clinical diagnostic biomarker for T2DM; ii) the effect of endogenous ketogenesis on T2DM; and iii) the effects of exogenous β-HB supplementation on patients with T2DM (Table II).

β-HB as a potential clinical diagnostic biomarker for T2DM. The level of β-HB in the blood may be associated with the risk of developing T2DM. Researchers from the Netherlands and Sweden found a positive correlation between fasting plasma β-HB levels and the incidence of T2DM in the general population without diabetes or impaired fasting glucose (142). However, Bae *et al* (143) followed up 453 patients with impaired fasting glucose from South Korea for 10.9 years and found that the incidence of T2DM was lower in patients in the high β-HB group (≥0.05 mmol/l). A previous study has shown that high levels of KBs are a marker of glucose metabolism disorders in prediabetes and an indicator of hyperglycemia in diabetes. Additionally, insulin sensitivity is negatively correlated with β-HB levels (144). The discrepancies between the conclusions made by Szili-Torok *et al* (142) and Bae *et al* (143) may be related to ethnicity, or they may stem from the key role of insulin as a KB regulator. Sufficient insulin secretion maintains low levels of KBs by inhibiting the expression of hormone-sensitive lipase (144). Based on the existing evidence, β-HB may serve as a novel predictive biomarker for the risk of developing T2DM. Additionally, β-HB may act as an early diagnostic biomarker for T2DM. Lucidi *et al* (145) conducted a study on 11 patients with T2DM and found that, compared with the normal control subjects, the blood β-HB levels were increased in patients with T2DM and the level of β-HB was higher in the afternoon than in the morning in these individuals. Garcia *et al* (146) employed nuclear magnetic resonance spectroscopy to measure plasma KBs in 373 patients with T2DM. The findings indicated an increase in all three types of KBs in patients with T2DM, with KBs levels showing a negative correlation with IR. In addition, elevated levels of β-HB may reduce the risk of developing complications in T2DM (146). The β-HB levels are increased in patients with T2DM and obesity (147,148). T2DM patients with impaired ketogenic function exhibit reduced insulin sensitivity and an increased risk of developing metabolism-related fatty liver disease (149). However, among patients with early-stage T2DM, those with intact ketogenic capacity have a lower risk of hepatic steatosis or fibrosis (150). A study by Liu *et al* (151) suggests that the higher the β-HB level, the better the renal function and the lower the risk of DKD. β-HB may also serve as a potential predictive biomarker for the therapeutic response in T2DM. Lee *et al* (152) showed that patients with T2DM with high initial serum β-HB levels are more likely to achieve well-controlled hemoglobin A1C levels after 6 months of anti-diabetic therapy.

Endogenous ketosis. Clinical studies indicate that lifestyle modifications, including dietary control and regular physical activity, can induce a state of NK that is beneficial for T2DM (153,154). Dietary control encompasses IF, CR and KD. IF refers to a period during which no food is consumed either daily or weekly. During the fasting period, there is a shift in metabolic pathways, transitioning from hepatic glucose metabolism to adipocyte-derived ketone metabolism. A study has shown that IF can alleviate IR in T2DM (155). Nuttall *et al* (156) conducted a 3-day fasting study on male patients with T2DM and observed that plasma β-HB levels increased (2.233±0.2 mM), while the insulin concentration remained unchanged. Currently available studies have not explained this result.

CR refers to a reduction of 25-50% in total daily caloric intake while providing adequate nutritional components such as essential amino acid and vitamins, to ensure that malnutrition does not occur. Steven *et al* (157) demonstrated that a 6-month intervention with a very low-calorie diet led to a decrease in fasting blood glucose levels in patients with T2DM. Vigili *et al* (158) showed that the baseline levels of β-HB increased in patients with T2DM, and after undergoing coronary angiography and an overnight fast, their β-HB levels increased further. Although this was a small-scale clinical trial, it also suggests that exploring and optimizing the preoperative fasting protocol for this group of patients is valuable.

KD is a formula diet characterized by a high proportion of fat, a low proportion of carbohydrates and appropriate amounts of protein and other nutrients. When undergoing a KD, the body metabolizes to produce increased levels of KB, which are utilized as an energy source (159). An extremely low-calorie KD can effectively reduce body weight in patients with T2DM and enhance glycemic control (160). In patients with T2DM, a HFD (85% fat, 15% protein and virtually carbohydrate-free) caused a sudden increase in β-HB only at the 8th h, which reached its maximum at the 10th h; however, this effect was far less significant than that of fasting. This mechanism may be associated with effectors other than insulin (156). Intervention with a KD can increase the plasma β-HB concentration in obese patients with T2DM (from a baseline of 0.22 mM to 0.44 mM during the intervention period). By contrast, in the group receiving combined intervention of KD and β-HB supplementation, the fasting β-HB concentration (baseline of 0.23 mM) reached a peak of 0.57 mM at 45 min and returned to the baseline level after 120 min. Both intervention approaches increased plasma insulin levels and C-peptide levels; however, they had no significant effects on blood glucose control, lipid metabolism or insulin sensitivity in muscle, liver and adipose tissues (161). This effect may be attributed to the conversion of β-HB into acetyl-CoA in β-cells. Acetyl-Coenzyme A then enters mitochondrial metabolism to generate ATP, which provides energy for the maintenance of β-cell function and may directly stimulate glucose-induced insulin secretion.

Therefore, the effects of IF, CR and KD on promoting β-HB to regulate body weight, fasting blood glucose levels, IR and other indicators in patients with T2DM remain inconsistent. Moreover, additional research evidence is required to clarify whether β-HB mediates these effects and to elucidate their underlying mechanisms. It is noteworthy that although dietary interventions can induce the human body to enter a state of

Table II. Therapeutic applications of β-HB in T2DM.

A, IF					
Authors, year	Human subject	Intervention method	Main findings	Mechanisms and indicators of change	(Refs.)
Arnason *et al*, 2017	Patients with T2DM (n=10)	2 weeks, fasting for 18-20 h daily	Short-term daily IF may be a safe and tolerable dietary intervention for patients with T2DM.	↓: Body weight, BMI, target morning blood glucose, fasting blood glucose, IR, CRP, caloric intake	(155)
Nuttall *et al*, 2020	Patients with T2DM (n=7)	3-day IF	β-HB increases with the duration of fasting.	↑: β-HB, IGFBP-1	(156)
Kramer *et al*, 2024	Overweight patients with early-stage T2DM (n=39)	6 weeks, fasting for 20 h every day.	IF improved β-cell function and IR in early-stage T2DM with overweight, accompanied by beneficial effects on obesity.	↓: HOMA-IR, HbA1c, body weight, waist, circumference; ↑: insulin secretion sensitivity index 2.	(186)
B, CR					
Steven *et al*, 2016	Patients with T2DM (n=30)	6 months of CR (43% carbohydrates, 34% protein and 19.5% fat, with an energy intake of 624 kcal per day).	CR diet reduces fasting blood glucose in patients with T2DM.	↓: Body weight, HbA1c, fasting blood glucose levels.	(157)
Vigili *et al*, 2017	Patients with T2DM before and after coronary angiography (n=11)	IF for 12-17 h in patients with T2DM.	Overnight fasting leads to inappropriate increase in β-HB in patients with T2DM.	↑: β-HB	(158)
C, KD					
Goday *et al*, 2016	Patients with T2DM (n=44)	Very low-calorie KD (15 g protein, 4 g carbohydrates, 3 g fat and 20 μg chromium, 0.8 g ginseng and 0.4 mg biotin).	The very low-calorie KD is safe and well-tolerated in patients with T2DM.	↓: Body weight, HbA1c, blood glucose	(160)

Table II. Continued.

C, KD					
Authors, year	Human subject	Intervention method	Main findings	Mechanisms and indicators of change	(Refs.)
Nuttall *et al*, 2020	Patients with T2DM (n=7)	3-day high-fat diet (85% fat, 15% protein, virtually carbohydrate-free).	KD leads to a significant increase in TAGs and NEFAs, which return to initial levels after 24 h.	↑: TAG, NEFA	(156)
Merovci *et al*, 2024	Overweight/obese patients with T2DM (n=10)	10-day intervention with a KD (15-25% protein, 5-10% carbohydrate and 70-80% fat).	It stimulates the production of ATP in β-cell mitochondria and significantly enhances the insulin secretion function.	↑: Plasma β-HB, insulin, and C-peptide	(161)
D, KE supplementation					
Soto-Mota *et al*, 2021	Patients with T2DM (n=21)	Continuously for 4 weeks, take 25 ml of KEM three times a day.	Exogenous KE supplementation induced significant reductions in all markers of blood sugar control.	↓: Fructosamin, HbA1c, average daily blood glucose.	(168)
Falkenhain *et al*, 2024	Patients with T2DM (n=18)	Single supplementation of KEM at a dose of 0.3 g/kg.	β-HB inhibits lipolysis in patients with T2DM, reduces FFA, decreases the supply of gluconeogenic amino acids and slightly increases insulin concentration.	↓: NEFAs, Met, Ser; ↑: β-HB	(170)
Jensen *et al*, 2020	Patients with T2DM (n=14)	Intravenous injection Na-DL-β-HB.	KB infusion improves working memory performance in patients with T2DM.	↑: Working memory	(171)
Baranowski *et al*, 2025	Patients with T2DM (n=15)	Acute and short-term (14-day) supplementation of exogenous ketone monoester.	Ketone monoester has no effect on plasma BDNF or cognition.	-	(172)

Table II. Continued.

D, KE supplementation					
Authors, year	Human subject	Intervention method	Main findings	Mechanisms and indicators of change	(Refs.)
Monteyne *et al*, 2024	Patients with T2DM (n=10)	Single supplementation of ketone monoester at a dose of 0.5 g/kg body weight.	β-HB delays glucose absorption in adults with T2DM, thereby reducing postprandial glucose concentrations.	↓: Glucose concentrations at 2 and 4 h post-prandially; ↑: plasma β-HB	(169)
E, KD + KE					
Merovci *et al*, 2024	Overweight/obese patients with T2DM (n=10)	10-day intervention with KE plus β-HB KE (8 g every 8 h).	It stimulates the production of ATP in the mitochondria of β-cells and significantly enhances the insulin-secreting function.	↑: Plasma β-HB, insulin, and C-peptide	(161)
F, Intravenous infusion					
Solis-Herrera *et al*, 2025	Patients with T2DM complicated by heart failure (n=36)	Intravenous infusion of β-HB at doses of 0.7, 1.6 and 3.2 mmol/l.	The myocardial benefits of β-HB are attributed to its ability to provide additional fuel to the heart without inhibiting MGU.	↑: Cardiac output, LVEF, and stroke volume	(173)

↑, increase; ↓, decrease; T2DM, Type 2 diabetes mellitus; IF, intermittent fasting; β-HB, β-hydroxybutyric acid; IGF-BP1, insulin-like growth factor binding protein 1; BMI, body mass index; IR, insulin resistance; CRP, c-reactive protein; HbA1C, hemoglobin A1C; CR, caloric restriction; KD, ketogenic diet; TAG, triacylglycerol; NEFA, non-esterified fatty acids; ATP, adenosine triphosphate; KE, ketone ester; BDNF, brain-derived neurotrophic factor; MGU, myocardial glucose uptake; LVEF, left ventricular ejection fraction.

NK, the adaptation period is extremely long and difficult to sustain (77). An excessively strict KD may also lead to adverse side effects (162,163).

Exogenous ketosis. Exogenous ketosis can increase the ketone levels in the body more rapidly and to a greater extent than endogenous ketosis (164). Current ketone supplements are roughly categorized into two major types: Ketone salts and ketone esters (KEs). Ketone salts consist of β-HB bound to minerals such as sodium, potassium and magnesium, whereas KE are ketones bonded to precursor molecules such as 1,3-BDO (165). The ketone monoester (KME) (R)-3-hydroxybutyl-(R)-3-hydroxybutyrate is a beverage that, upon ingestion, is metabolized by intestinal esterases into β-HB and 1,3-BDO in equal proportions. Both compounds then enter the portal circulation where the latter is transformed into β-HB in the liver (166). Compared with endogenous ketosis, exogenous KB supplementation can cause a sharp increase in blood β-HB levels without the need for prolonged fasting or adherence to a KD (167). Long-term exogenous supplementation of β-HB can improve blood glucose control in patients with T2DM. Soto-Mota *et al* (168) found that administering 25 ml of exogenous ketone three times a day for 4 weeks reduced the levels of glycemic control markers in patients with T2DM. However, the effect of a single-dose intervention on blood glucose control remains controversial. In a study by Monteyne *et al* (169), 10 patients with T2DM were enrolled. These patients were instructed to take (R)-3-hydroxybutyl-(R)-3-hydroxybutyrate orally at a dose of 0.5 g/kg, 30 min before meals. The results showed that the plasma β-HB concentration in the patients increased from 0.3±0.03 to a peak of 4.3±1.2 mmol/l and their postprandial blood glucose levels were significantly reduced. Oral administration of KME before meals can safely induce ketosis in T2DM patients and lower postprandial blood glucose, providing a new metabolic intervention approach for postprandial blood glucose management in patients with T2DM. Falkenhain *et al* (170) found that 30 min after a single oral administration of 0.3 g/kg KME, blood β-HB levels increased from 0.2±0.1 mM to 1.5±0.8 mM, reached a peak of 1.7-1.8±0.6 mM at 60-90 min and then decreased to 0.8±0.4 mM at 180 min. Additionally, serum insulin levels increased, while lipolysis was inhibited and gluconeogenic precursors were reduced; however, there was no effect on fasting blood glucose levels. This discrepancy may be related to the dose as a relatively small oral dose was used in the study.

Intravenous injection of β-HB (0.22 g/kg/h for 120 min) have been shown to improve working memory in patients with T2DM (age, 65±4 years) (171). By contrast, a study by Baranowski *et al* (172) found that acute (0.3 g/kg) or short-term (15 g, 14 days) oral β-HB supplementation had no beneficial effect on the cognitive function of patients with T2DM (age, 30-70 years). These discrepancies may be related to the duration of β-HB supplementation, the dosage administered and the methods used to assess cognitive function. A study by Solis-Herrera *et al* (173) found that intravenous infusion of β-HB exerts a clear 'threshold effect' on cardiac protection in patients with T2DM complicated by heart failure. When the plasma β-HB concentration is ≥1.6 mmol/l, β-HB provides energy substrates for the heart without inhibiting myocardial glucose uptake, thereby improving left ventricular systolic function. A 2-week KE intervention was shown to increase the circulating β-HB level by ~10-fold in patients with T2DM complicated by heart failure with preserved ejection fraction and was accompanied by improvements in resting and exercise hemodynamic status (174). This indicates that β-HB supplementation exerts a protective effect on cardiovascular function in patients with T2DM. The pharmacology and safety of KEs have been intensively investigated in animals (175), healthy humans (176) and patients with T2DM (168). However, most ketone salts are racemic (with mixed chirality) and L-β-HB is not a naturally occurring substance in the human body. For ketone salts existing in the D/L form, if the amount of L-chiral isomer is higher than the D-chiral isomer, it tends to prolong the time originally required to induce ketosis (177). Ketone salts have a relatively simple manufacturing process and low cost, but they are prone to causing gastrointestinal side effects (178). By contrast, KEs have much greater safety and tolerability in the human body than ketone salts (179); however, they need to be metabolized by the liver first before being degraded into acids for absorption.

6. Conclusion

With the prevalence of T2DM and its related complications on the rise, this condition has become a pressing global health issue that poses a notable risk to human health and well-being. The scientific community has been diligently researching new preventative and therapeutic approaches. Recent findings indicate that β-HB is a predominant KB with preventive and therapeutic potential, functioning as an energy metabolite and signaling molecule across various pathologies, including aging, cancer, neurological disorders and T2DM. Current clinical studies have shown a correlation between circulating β-HB levels and the pathological progression of T2DM, suggesting its potential utility as a diagnostic indicator for the disease. Additionally, β-HB, whose levels are elevated either through endogenous ketosis or exogenous supplementation, possesses pharmacological activity. This activity may be associated with the pathogenesis of T2DM and β-HB thus holds promise as a potential therapeutic agent for the prevention and treatment of T2DM. Animal and cellular studies have explained the potential mechanisms of β-HB in the pathological progression of T2DM. β-HB exerts its effects by modulating glucose and lipid metabolism, safeguarding pancreatic β-cells and mitigating IR. Additionally, it acts as a signaling molecule that promotes cellular protein homeostasis, inhibits oxidative stress and inflammation, alleviates ERS and regulates mitochondrial biosynthesis, autophagy, apoptosis and other pathways to combat T2DM and its multi-organ complications.

7. Limitations and future directions

At present, the optimization of pathological diagnostic indicators for T2DM, in-depth analysis of its pathogenesis and the development of clinical drugs still face a number of unsolved challenges. Particularly in the research field related to β-HB, the limitations of existing evidence have significantly restricted its clinical translational application. There remain numerous pending issues to be addressed regarding the pathological

diagnostic indicators of T2DM, as well as in-depth mechanistic research and the development and application of clinical drugs.

At the clinical level, studies on the detection of β-HB levels in patients with T2DM are not only scarce in quantity, but the existing studies also generally suffer from limitations such as small sample sizes and inconsistent detection methods. These issues result in the inability to clearly define the threshold value for T2DM at present, making it difficult to use β-HB as a reliable indicator for clinical diagnosis or disease condition assessment. In the field of mechanistic research, the exploration of the mechanism by which β-HB acts on T2DM and its complications remains confined to *in vitro* cell experiments and animal model studies. There is a lack of mechanism validation based on human clinical samples, leading to a distinct 'translational gap' between laboratory conclusions and practical clinical applications. Meanwhile, existing animal and cellular studies have notable technical limitations. Most studies focus on the detection of plasma β-HB concentration, while neglecting the differences in the expression and distribution of β-HB in T2DM target organs such as the liver, brain and kidneys, making it impossible to reveal its 'organ-specific regulatory effect'. Although current studies have demonstrated that β-HB is generally considered to exert metabolic benefits in most organ systems of patients with T2DM, its effect of inhibiting GLP-1 secretion *in vitro* (where GLP-1 is a first-line clinical target for glucose lowering) creates a significant contradiction (180). In GLUTag cells (mouse colonic endocrine cells), both low-dose (0.01 mM) and high-dose (100 mM) β-HB inhibit glucose-induced GLP-1 secretion, while intermediate doses have no effect. In human jejunum-like monolayer cells, β-HB at a dose of 10 mM inhibits glucose-induced GLP-1 secretion (180). This result is somewhat pharmacologically puzzling. Even though the local level in the intestinal mucosa is close to 10 mM (181), 100 mM is still far beyond the physiological concentration *in vivo*. However, current experimental studies only represent results at the cellular experimental level and the effect of β-HB on GLP-1 in the digestive system under *in vivo* conditions remains unknown. Additionally, existing experiments involve only short-term exposure with no consideration given to the impact of time. Additionally, Wang *et al* (182) used resonance Raman scattering technology and found that intraperitoneal injection of β-HB improves mitochondrial function in T2DM model mice, thereby alleviating pathological phenotypes of T2DM such as elevated blood glucose, IR, systemic inflammation and multi-organ damage. However, from an overall perspective, the experimental methods are relatively singular and there is a lack of application of precise tools such as omics technologies (such as combined metabolomic and transcriptomic analysis) and plasmid transfection (such as for the overexpression/silencing of specific genes). This makes it difficult to systematically analyze the interaction between β-HB and other signaling molecules as well as the mechanism of multi-organ crosstalk.

In terms of regulatory strategies for the source of β-HB, the induction of endogenous ketosis (such as IF, CR, KD and exercise intervention) can increase circulating β-HB levels, thereby providing a potential direction for the treatment of T2DM and its complications. However, existing studies have not fully considered the impact of the 'time factor'. For instance, questions such as whether long-term endogenous ketosis can trigger metabolic adaptation in the body and the differences in the regulatory effect of β-HB between different intervention durations (such as short-term vs. long-term KD) remain unclear. Additionally, most intervention strategies utilize a single dose of β-HB (such as intraperitoneal injection of β-HB salts at a fixed concentration). Comparative studies investigating different doses and various administration routes (such as intraperitoneal injection vs. oral administration) have not been conducted, making it impossible to clarify the 'dose-effect relationship' through which β-HB exerts its effects and the optimal administration regimen. Notably, prolonged moderate-intensity exercise or high-intensity interval exercise can significantly increase the oxidation of fatty acids in muscles, thereby promoting the production of KBs in the liver. These KBs are then oxidized as an energy source during exercise and increase significantly during the post-exercise recovery period; the skeletal muscles adapted to exercise training exhibit a higher capacity to utilize KBs (70). During exercise, the oxidation of KBs in the muscles involved in the exercise increases (183,184). Scientific exercise, as an effective method for ketone elevation and improvement of T2DM, has received little attention in research. Only Wang *et al* (185), using untargeted metabolomics, found that β-HB levels were decreased in the cardiac tissue of Sprague Dawley rats with T2DM. However, 8 weeks of aerobic exercise increased β-HB levels and improved cardiac function in these T2DM rats. This suggests that β-HB may be a key target for aerobic exercise to improve cardiac function in T2DM.

Exogenous β-HB supplementation faces practical challenges related to dosage forms. Ketone salt preparations, though easily accessible, may cause gastrointestinal adverse reactions (such as nausea and diarrhea) and excessive mineral load in the body (such as sodium and potassium overload) and KE preparations carry potential risks of excessive L-β-HB intake, while pharmaceutically active pure D-β-HB acid preparations are currently scarce in market supply and expensive, making them unable to meet the needs of basic research and clinical translation. More notably, the mechanisms of action between endogenously induced β-HB production and exogenous β-HB supplementation in improving T2DM are both related and distinct. The core regulatory networks and interactive relationships between the two remain unclear, which limits the precise design of β-HB intervention strategies. At the level of drug development, as a potential drug, β-HB still lacks comprehensive basic research on its quality standardization (including purity and impurity control), pharmacodynamics (including duration of action and the dose-effect relationship) and pharmacokinetics (including absorption, distribution, metabolism and excretion patterns), which further hinders its drug development process.

In summary, β-HB has demonstrated clear potential in the prevention and treatment of T2DM and its complications and is expected to become a key focus in the fields of healthcare and pharmaceutical research in the future. However, current research must prioritize breaking through core bottlenecks such as the standardization of clinical detection, the clinical translation of mechanistic research, the optimization of intervention strategies and the development of pharmaceutical

dosage forms. Through the integration of multidisciplinary technologies and multi-center collaboration, β-HB research can be advanced from basic research to clinical application. Ultimately, this will provide a novel and efficient metabolic intervention regimen for the prevention and treatment of T2DM, contributing to the development of the human health industry.

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Authors' contributions

XYD contributed to conceptualization, funding acquisition, project administration, original draft writing as well as reviewing and editing the manuscript. JBW, LW, KL, HYG, MYW, QYZ and RNH contributed to reviewing and editing the manuscript. WHW contributed to conceptualization as well as reviewing and editing the manuscript; WHX contributed to funding acquisition, project administration as well as reviewing and editing the manuscript. Data authentication is not applicable. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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