

# Insulinotropic Effects of Whey: Mechanisms of Action, Recent Clinical Trials, and Clinical Applications

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## Key Words

Insulin · Whey · Hyperglycemia · Diabetes

## Abstract

**Background:** The insulinotropic effect of whey protein is not fully understood and has clinical implications in the regulation of chronic and acute hyperglycemia. **Summary:** This review describes the composition of whey protein and potential mechanisms through which whey exerts an insulinotropic effect, including increasing the gastric emptying rate, effect on incretin hormones particularly gastric inhibitory peptide and glucagon-like polypeptide-1, and whey's role as a dipeptidyl peptidase IV inhibitor. Recent clinical evidence on the use of whey protein concentrate, isolate and hydrolysate in the management of type 2 diabetes and in the acute care adult population is reviewed. **Key Messages:** The mechanism through which whey protein exerts its insulinotropic effect is multifactorial. **Increasing evidence supports the potential use of whey protein in medical/nutritional therapy to manage glycemia;** however, additional research is needed to determine the most appropriate dose, form and delivery method for whey supplementation.

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## Introduction

Recently, increasing research has focused on the potential insulinotropic effect of whey protein in healthy individuals and patients with hyperglycemia. Studies show whey protein consumption has a positive effect on the glycemic response and increases insulin sensitivity [1–5]. Effective doses of whey protein necessary to induce beneficial shifts in postprandial glucose levels or insulin secretion varies between 10 and 55 g when provided with a meal or as a preload [3, 6]. Improved postprandial glycemia enhances overall glucose homeostasis in patients with type 2 diabetes mellitus (T2DM) [2, 7], possibly delaying the need for medical treatment [2, 8]. Reductions seen in postprandial glycemia resulting from whey supplementation are similar to what would be expected from pharmacological agents [3]. Recently, studies have concluded that whey preloads should be considered as a long-term management strategy in patients with T2DM [9, 10]. However, in order to be effective, whey protein must remain a continual part of the diet [6]. Additional research shows potential benefits of whey in the management of acute hyperglycemia [11–14].

Hyperinsulinemia has been linked to obesity through its role in fatty acid synthesis and decreased adipose tissue fatty acid release [4]. However, increased insulin induced

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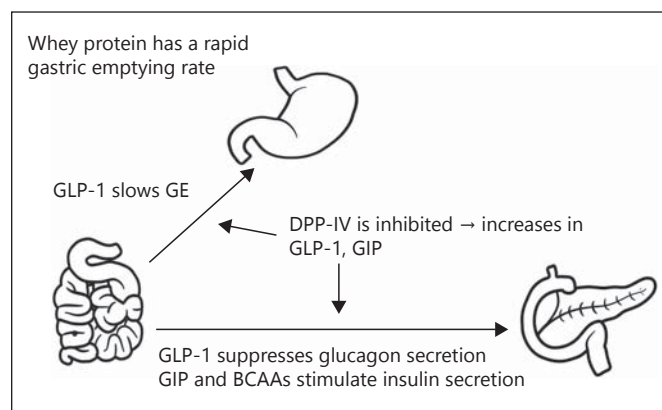
by whey protein intake does not appear to increase fat mass [4]. The purpose of this review is to examine plausible mechanisms through which whey protein exerts its insulinotropic effect, reviewing evidence of its role in the management of glycemia in chronic and acute hyperglycemia. Initially, the composition of whey protein and its potential insulinotropic mechanism will be described. Thereafter, recent clinical trials that evaluated the insulinotropic effect of whey in various forms and types of hydrolysis in patients with type 2 diabetes and those with acute hyperglycemia will be summarized.

### Composition of Whey Protein

Whey protein, found in the liquid portion of milk, is removed from casein in the cheese-making process. Whey is commercially available in a variety of forms including whey protein concentrate (WPC), whey protein isolate (WPI), reduced lactose whey, partially and extensively hydrolyzed whey and demineralized whey protein [6]. Studies have yielded equivocal results regarding the effective form of whey protein. The mechanism by which whey improves the glycemic response remains speculative with the effective dose varying by study, dependent on whey type and experimental methodology.

Whey protein consists of  $\beta$ -lactoglobulin (45–57%),  $\alpha$ -lactalbumin (15–25%), immunoglobulin (10–15%), glycomacropeptide (10–15%), bovine serum albumin (10%), lactoferrin (~1%) and lactoperoxidase (<1%) [4, 15, 16]. Whey is a complete protein, meaning it contains all of the essential amino acids. Whey is also considered a high-quality protein based on measures of protein quality in humans and animals. Haraguchi et al. [17] evaluated the biological and biochemical properties of whey and found whey, as a blend of WPC, partially hydrolyzed whey and WPI, has a significantly higher protein efficiency ratio, net protein ratio and true digestibility when compared with casein or a casein/whey blend, as measured in rats, which the authors noted was comparable to results in humans. The rat model also showed positive significant differences for albumin, total protein, total cholesterol and glucose concentrations with whey compared to casein or the blend.

Whey protein has bioactive peptides and amino acids that are released during hydrolysis [15]. These components are thought to be responsible for much of the functional benefits of whey protein on human health [15, 18]. The degree and type of hydrolysis impact the effects of whey protein [10, 19–26]. Whey protein is rich in the branched chain amino acids (BCAAs) isoleucine, leucine



**Fig. 1.** MoA 1: bioactive peptides from digestion or hydrolyzed whey activate incretin hormone release. MoA 2: rapid digestion of whey results in a rapid rise in amino acids (BCAAs, in particular), which results in increased insulin release. MoA 3: amino acids and peptides from hydrolyzed (in vitro hydrolysis from pepsin or trypsin or in vivo digestion) whey inhibit DPP-IV to stop degradation of GIP and GLP-1. MoA = Mechanism of action.

and valine, which along with its bioactive peptides may indirectly affect the glycemic response [6]. In an in vitro study of a mouse pancreatic islets incubated with serum from healthy humans following a whey and carbohydrate meal compared to a control, a combination of BCAAs and the amino acids lysine and threonine increased insulin secretion by 270% [27]. While the amino acid profile may contribute to its insulinotropic effects, the form of the protein (i.e., concentrate, isolate, hydrolysate, etc.) has varying effects on insulin secretion [3].

### Mechanisms of Action

Recently, several studies have found that consuming whey prior to or with a meal significantly decreased postprandial glycemia and improved the insulin response [5, 9, 19, 21, 23, 28]. Several mechanisms have been proposed to explain how whey is able to exert these effects. However, the answer likely involves multiple, interconnected pathways (fig. 1).

Early investigative work by Nilsson et al. [29] examining the insulinotropic effect of milk, and whey specifically, attributed the benefits to the bioactive peptides or amino acids. In healthy subjects, both milk and whey-based test meals resulted in lower postprandial glucose areas under the curve (AUCs) than a white bread reference meal (–62 and –57%, respectively). However, a whey meal led to significantly higher AUCs for insulin (90%)

and gastric inhibitory peptide (GIP, 54%). The postprandial amino acid response was also more substantial for the whey meal, which included the highest incremental rise in BCAAs – known stimulators of insulin secretion.

Nilsson et al. [29] proposed that intestinal digestion of whey results in bioactive peptides or specific amino acids, activating the release of incretin hormones. However, it was noted that the insulinotropic effect of whey could not be due to the incretin system alone. The authors hypothesized that the postprandial increased insulin response with whey protein could be attributed to a more rapid digestion of whey protein than casein.

### *Gastric Emptying*

In a review of the relationship between gastric emptying, postprandial glycemia and incretin hormones, Marathe et al. [7] hypothesized that gastric emptying is a key regulator of postprandial glycemia. The review noted postprandial glycemia is an important indicator of overall blood glucose control, particularly in patients with T2DM who have mild to moderately elevated hemoglobin A1c (HbA1c).

In a review of dairy proteins, Bendtsen et al. [16] described the effect of the acidic environment of the stomach on protein types noting whey remains in a liquid form, while casein coagulates. This coagulation slows the release of amino acids and gastric emptying of casein. Whey proteins have been classified as ‘rapid release’ proteins, because they empty from the stomach and are metabolized faster than many other proteins [6, 16, 27, 30]; however, the literature is inconsistent. Calbet and Holst [31] reported that WPI and hydrolysate and casein isolate and hydrolysate, when given in isoenergetic solutions with similar energy density, had gastric emptying rates that were similar in a small study of 12 healthy men receiving 60 g of protein supplements. Findings from Power et al. [26] also supported the notion that gastric emptying was not affected by hydrolysis of proteins.

Whey’s ability to quickly stimulate insulin secretion may be due to the rapid postprandial protein digestion of whey and subsequent rapid rise in amino acids, which are second only to glucose in promoting insulin secretion [6, 16]. More specifically, it may be the BCAAs that lead to the rapid insulin response, as insulin secretion rates are affected differently depending on the amount and type of circulating amino acids [6].

### *Incretin Hormones*

Postprandial glycemia may be determined by the gastric emptying rate and the action of the incretin hormones, glucagon-like polypeptide-1 (GLP-1) and GIP.

Both of these incretin hormones have strong insulinotropic effects, though they work through different mechanisms – GIP stimulating insulin release and GLP-1 exerting a negative feedback mechanism on gastric emptying [4, 7, 23, 27, 32–34]. GLP-1 appears to be particularly important in the management of postprandial glycemia in patients with T2DM, since GIP is diminished [35, 36]. Other hormones linked to appetite regulation, including cholecystokinin (CCK) and peptide YY, also appear to influence the gastric emptying rate [7]. The rates of gastric emptying and incretin hormone release have a comingled effect, as gastric emptying influences the rate of incretin hormone secretion [32]. Marathe et al. [7] asserts that incretin effect is greater when the gastric emptying rate is faster and that the gastric emptying rate is responsible for about 35% of the incretin response in both healthy individuals and those with T2DM.

Amino acids and peptides may directly facilitate the increase of gut-derived incretin hormones, GIP and GLP-1 [27]. These incretin hormones are partially responsible for increasing insulin secretion from pancreatic cells in response to glucose; decreasing glucagon release from the pancreas; enhancing the amount and lifespan of the pancreatic  $\beta$ -cells, which store and release insulin; delaying gastric emptying and helping to regulate appetite [23, 37, 38].

Whey protein has consistently been shown to increase GIP, but whey’s effect on GLP-1 is more variable. Whey significantly increased both GIP and GLP-1 in a model in which mouse pancreatic islet cells were incubated with serum from healthy humans consuming whey or a control (white bread) [27]. The increase in GIP led to increased insulin secretion from the cells incubated with serum taken postprandial from humans who consumed whey. The authors noted in their previous studies that whey protein only stimulated GIP secretion [8, 29, 39]. Bohl et al. [40] found that casein increased the postprandial GLP-1 response compared to whey protein in a study on obese adults given 60 g of whey or casein protein with 65 g of milk fat with either high or low medium-chain saturated fatty acids over 12 weeks. Despite increased GLP-1 in the casein-supplemented group, there were no significant differences in postprandial glucose or insulin. Increases in GIP secretion were not significantly different between the 2 proteins. The high fat content of the test meal may have blunted whey’s effect on the incretin response.

Protein hydrolysis and the liberation of amino acids and di- and tri-peptides may contribute to the rate of release of incretin hormones. The impact of whey on the incretins is inconsistent likely due to vast differences in methodolo-

gies, as results vary between lean and obese individuals, the method of protein delivery and the type and degree of hydrolysis. Calbet and Holst [31] found that both whey and casein protein hydrolysates elicited 50% more ( $p < 0.05$ ) gastric secretions and greater GIP release than their whole-protein counterparts during the first 20 min of gastric emptying after subjects were fed test formulas through a nasogastric tube. In healthy, obese men, intraduodenal infusions of whey protein hydrolysate (WPH) over 60 min resulted in significant treatment  $\times$  time interactions with plasma CCK, GLP-1, GIP, glucagon, insulin and glucose [41]. When lean and obese subjects in the same study were compared, CCK, GLP-1 and glucagon AUCs did not differ; however, GIP AUC was significantly lower in obese than in lean subjects and insulin was higher in obese compared to lean subjects. The author's speculated that even in the healthy obese people, the insulin-cretin axis may be affected early since the GLP-1 response was similar in both lean and obese participants while the GIP response was diminished. In contrast, Mortensen et al. [10] found GIP incremental AUC (iAUC) to be significantly lower at both 60 and 120 min for the WPH compared to the WPI. However, GLP-1 and glucagon were both higher at 30 min postprandial for the WPH than WPI. Power-Grant et al. [25], in an in vitro study, noted that intact whey protein stimulated GLP-1 secretion compared to control ( $p < 0.05$ ), with no significant effect seen for WPH.

#### *Dipeptidyl Peptidase-IV Inhibitors*

Whey-derived amino acids and peptides have the ability to inhibit the action of dipeptidyl peptidase-IV (DPP-IV) [23, 25]. DPP-IV rapidly degrades GIP and is responsible for over 95% of the rapid degradation and inactivation of GLP-1 [42–44]. Mannucci et al. [45] found that DPP-IV levels are inversely correlated with age and positively correlate with HbA1c, but remain unaffected by BMI. DPP-IV activity is positively correlated with hyperglycemia, as measured by HbA1c, in patients with both type 1 DM and T2DM. Levels of DPP-IV were found to be significantly higher in patients with HbA1c  $>8.5\%$  compared with patients who had impaired glucose tolerance (IGT) or normal glucose tolerance (NGT). Patients with mild hyperglycemia did not have significant increases in DPP-IV activity compared to patients with IGT or NGT. While DPP-IV activity is known to reduce levels of GIP and GLP-1, Mannucci et al. [45] asserts that it cannot be assumed that this is the mechanism by which DPP-IV levels are correlated to HbA1c, as the correlation was also found in type 1 DM patients whose dysfunctional  $\beta$ -cells do not secrete insulin. Study results showed that increased

DPP-IV activity only occurs in times of significant hyperglycemia. As such, the decrease in GLP-1 response may be due to decreased release of the incretin hormones in the early stages of diabetes. However, the exact mechanism of action (MoA) remains unclear.

Silveira et al. [46] determined that for whey protein to inhibit DPP-IV activity, peptides must be hydrolyzed through either in vitro hydrolysis or in vivo digestion. Undigested whey protein does not appear to inhibit DPP-IV activity. However, the type of hydrolysis, more specifically the type of protease used in hydrolysis, affects the DPP-IV inhibitory activity.

Hydrolysis of whey protein by pepsin and trypsin results in a release of peptides that inhibit DPP-IV in in vitro and in vivo models [22, 23, 46]. Silveira et al. [46] found peptides derived from the  $\beta$ -lactoglobulin fractions of whey protein hydrolyzed by trypsin inhibit DPP-IV. In another in vitro study, trypsin-treated  $\beta$ -lactoglobulin dose-dependently significantly decreased DPP-IV activity; however,  $\beta$ -lactoglobulin ingestion that was not trypsin-treated had no effect [47]. A more recent in vitro study by Power-Grant et al. [25] reported that the effectiveness of whey hydrolysates on DPP-IV inhibition increased with higher degrees of hydrolysis. Whey hydrolysis of both 32% (DH32) and 45% (DH45) were significantly more potent than non-hydrolyzed WPC in decreasing DPP-IV activity. The results of the inhibitory concentrations of the hydrolysates were similar to previous studies [22, 48, 49]. In a mouse model, trypsin-hydrolyzed  $\beta$ -lactoglobulin was also found to lower blood glucose [42].

#### **Hydrolyzed versus Intact Whey**

Studies comparing protein hydrolysates with intact proteins have predominantly found hydrolyzing protein results in an improved glycemic response in healthy and T2DM subjects. Nongonierma et al. [24] tested 3 different WPHs against intact whey proteins to determine their insulinotropic properties in the presence of glucose using BRIN-BD11 pancreatic beta cells. Results showed significantly elevated insulin secretion values for the WPHs compared to the intact whey. Likewise, the authors found lower free amino acids correlated with a decrease in insulin secretion. The WPH inducing the highest increase in insulin secretion had a significant amount of BCAAs. However, when the WPH-1 was fractionated, 2 fractions (Strata-X and 2 kDa permeate) had significantly higher free amino acids, but only resulted in a moderate insulin

response. Researchers concluded that the insulinotropic properties of WPHs may be correlated not only with free amino acids, but also available peptides. In an earlier study, Mortensen et al. [10] noted that a WPH that increased serum insulin at both 30 and 480 min postprandial contained a significant portion of di- and tri-peptides. The authors asserted that whey protein could be used as a 'potential nutritional protein source(s) to improve glucose homeostasis' and that WPI and WPH may have 'nutraceutical benefits in the treatment of T2DM, especially in long-term diabetes where the glucose-sensing capacity of the pancreatic  $\beta$ -cells is reduced'.

Power-Grant et al. [25] assessed intact whey protein versus whey protein ingredients at 32% hydrolysis (DH32) and 45% hydrolysis (DH45) on the insulin response. DH32, with higher concentrations of free arginine and lysine, amino acids previously shown to significantly increase insulin secretion, had an insulinotropic effect in vitro. The author's suggest the insulinotropic effects while, as yet unknown, are not solely tied to the degree of hydrolysis, but may instead be related to the presence of insulinotropic amino acids or peptides.

### Insulinotropic Effects of Whey

#### *Insulinotropic Effects of Whey Protein: Acute Care*

Insulin resistance and hyperglycemia are common in the acute care setting and significantly increase the risk of complications and death [11]. Perrone et al. [12] examined the effect of a whey and carbohydrate (14 and 84%, respectively) beverage on the acute phase response and insulin resistance after a scheduled cholecystectomy or inguinal herniography. The results showed an improved acute phase response to the trauma with significantly lower postoperative C-reactive protein and, while albumin dropped in both groups, only the control group's decrease was significant. Mean changes between preoperative and postoperative HOMA-IR (control:  $4.8 \pm 1.1$ , whey/CHO:  $-2.5 \pm 1.5$ ;  $p = 0.001$ ), insulin (control:  $15.5 \pm 3.8$ , whey/CHO:  $-8.8 \pm 4.6$ ;  $p < 0.00$ ) and glycemia (control:  $30.0 \pm 7.3$ , whey/CHO:  $1.6 \pm 12.1$ ;  $p = 0.036$ ) were all significantly lower in the group receiving the whey and carbohydrate beverage.

In a study by de Aguilar-Nascimento et al. [13], the effect of whey protein on the acute phase response in elderly patients after an acute ischemic stroke was evaluated. Patients were randomized to a standard hydrolyzed casein-based enteral formula or an isocaloric and isonitrogenous formula made of hydrolyzed whey protein.

Glucose changes were not significant between groups or within groups between day 1 and 5. However, glucose tended to increase in the whey group from  $132 \pm 19$  mg/dl at day 1 to  $139 \pm 18$  mg/dl on day 5, while the casein group increased from  $148$  mg/dl on day 1 to  $214 \pm 43$  mg/dl on day 5.

Kaido et al. [14] evaluated the effect of hydrolyzed whey protein in an immunomodulating diet (IMD) compared to a traditional elemental diet in the first 24 h after a live donor liver transplant in 76 adult patients. Fasting blood glucose at postoperative day 7, a secondary end point, was significantly less in the hydrolyzed whey-enriched IMD group compared to control ( $125 \pm 30$  vs.  $145 \pm 36$ ,  $p = 0.005$ ).

#### *Insulinotropic Effects of Whey: Type 2 Diabetes*

Whey protein administration both as a preload and with a meal resulted in significant insulinotropic effects and reductions in glycemia. Frid et al. [8] studied the effect of whey protein on the postprandial glucose and insulin response in subjects diagnosed with T2DM. Subjects were given a high glycemic index breakfast and lunch with 27.6 g whey protein added to the meals one day and, alternatively, ham and lactose were added on another day (protein and lactose levels were matched at meals). At 180 min postprandial, glucose AUC was significantly lower and insulin AUC and GIP were significantly higher post whey ingestion. Also, a significant treatment  $\times$  time response for serum insulin incremental change was found in the test meal group through 60 min for the breakfast and lunch meals.

Ma et al. [9, 28] conducted 2 trials on type 2 diabetics using protein preloads to assess gastric emptying, the incretin response and the effect on postprandial glycemia. In the first study [9], 55 g of whey protein was either given as a preload or with a meal. Gastric emptying was significantly slower when whey was added either as a preload or with the meal compared to the control. The iAUC for serum glucose was lower for both whey preload (363.7 mmol/min) and whey in meal (406.3 mmol/min) compared with no whey (734.9 mmol/min,  $p < 0.005$  for both). The AUC for insulin, GLP-1, GIP and CCK were significantly greater. Ma et al. [28] decreased the preload to 25 g of whey in a second study and found significantly decreased postprandial blood glucose, peak blood glucose and gastric emptying compared to placebo. These results persisted after 4 weeks of whey preload prior to each of the 3 main meals, though peak blood glucose reduction was no longer significant. Interestingly, despite the additional calories from the added whey, energy intake and BMI did not differ between the 2 groups at baseline or after 4 weeks of whey supplementation.

Hydrolysis of whey protein may have further positive effects in the management of T2DM. Gaudel et al. [20] investigated the effect of WPH on glucose-induced insulin secretion in a pancreatic beta-cell line over 8 weeks in mice. WPH improved blood glucose clearance, decreased hyperinsulinemia and increased beta-cell secretion of insulin in the ob/ob mice. The study concluded that WPH may be useful in patients with insulin resistance or T2DM. Another study in adult men with T2DM concurred with their conclusion finding that patients who received 0.4 g/kg WPH did not experience the significant rise in blood glucose typically seen postprandial in patients with T2DM, an effect they attributed to continuous delivery of glucose into the cells induced by the elevated insulin levels [21]. Preloads of 0.2 g/kg WPH and 0.4 g WPI resulted in postprandial glucose and insulin changes similar to those found in pre-diabetic patients, while 0.4 g/kg WPH dose patients experienced changes in blood glucose and insulin levels that were comparable to those seen in non-diabetic subjects after an OGTT.

Currently, orally administered inhibitors of DPP-IV, gliptins, are used in pharmacotherapy for the treatment of T2DM to lower fasting and postprandial blood glucose [49, 50]. Gliptins stimulate insulin secretion when blood glucose is elevated, helping reduce weight gain and hyperglycemia [49, 51]. When WPH was combined with sitagliptin, whey protein further enhanced the effect of the gliptin as a DPP-IV inhibitor *in vitro* [49]. The same study also found WPH independently inhibited DPP-IV activity, though the effect was less potent than the combined therapy. A combined therapy that included a preload of 25 g WPI 60 min post administration of another gliptin – vildagliptin – in patients with T2DM on metformin resulted in significant increases in the levels of GIP, GLP-1 and decreases in postprandial glycemia and slower gastric emptying [52]. The study also tested WPI preloads again in the same patients on metformin without vildagliptin and found significant reductions in the glycemic peak, increased insulin and total and intact GLP-1 and GIP compared to control.

## Conclusion

A growing body of evidence supports the insulinotropic effects of whey protein. Studies employ differing delivery methods (i.e., preload, as a stand-alone supplement, with a meal), varying doses (4.5 to over 50 g) and forms (i.e., concentrate, isolate, hydrolysate, etc.) of whey protein. While varying methodologies have yielded in-

consistent results regarding the degree of the insulinotropic effect and subsequent glycemic response, the majority of data support the addition of whey protein to the diet for an improved glycemic response in patients with acute and chronic hyperglycemia. However, before whey supplementation becomes standard clinical practice, additional research should focus on long-term trials where HbA1c can be observed to understand the impact of whey's insulinotropic effects on glycemia over time. The ability of whey to increase insulin without increasing adipose tissue deposition is also not yet understood.

There is limited research to suggest whether whey provided as a preload or with a meal would have had a greater effect on postprandial glycemia. As whey protein is available in a variety of forms, increased data on the forms (WPC, WPI, WPH, etc.) and doses of whey are needed. In addition, no studies were found that considered different methods of hydrolysis (microbial versus enzymatic and the type of enzyme used). Finally, in order to be translatable, research should also focus on palatable doses of whey – noting that hydrolysis certainly affects the taste of whey protein.

While research is still needed, there appears to be significant potential for the use of whey protein as a preventative measure in individuals who are at risk of developing T2DM. Also, whey could function as an adjunctive or stand-alone therapy for the treatment of acute and chronic hyperglycemia, as whey protein is a low-cost and low-risk option. While the long-term use of whey protein in the management of hyperglycemia requires further study, clinicians should begin investigating ways to incorporate whey into their current practices to help prevent or treat T2DM and manage acute hyperglycemia.

## Disclosure Statement

The author has nothing to disclose.

## Author's Contributions

R.L.A. was responsible for the literature review and development of the review. K.S.B. provided editorial feedback and assisted in outlining the review's content.

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