Journal of Nutrition and Food Security
Shahid Sadoughi University of Medical Sciences
School of Public Health
Department of Nutrition
Shahid Sadoughi University of Medical Sciences
School of Public Health

eISSN: 2476-7425 pISSN: 2476-7417 JNFS 2025; 10(1): 178-193 Website: jnfs.ssu.ac.ir

The Effect of Whey Protein Consumption on Postprandial Glucose, Insulin and Incretin Responses in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis of Acute-Term Controlled Clinical Trials

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ARTICLE INFO

SYSTEMATIC REVIEW and META-ANALYSIS

Article history:
Received: 23 Jul 2023
Revised: 28 Nov 2023
Accepted: 21 Dec 2023

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Keywords:
Whey protein;
Postprandial;
Glucose;
Insulin;
Type 2 diabetes.

ABSTRACT

Background: Postprandial hyperglycemia is an important factor which contributes to glycemic status and complications of diabetes mellitus. This was a systematic review and meta-analysis that was conducted to evaluate the overall effect of whey protein consumption on postprandial glucose, insulin, and incretin hormone responses in patients with type 2 diabetes mellitus (T2DM). **Methods**: Web of Science, Cochrane Library, EMBASE, PubMed, and Scopus databases were searched to find acute-term controlled clinical trials investigating the effect of all types of whey proteins on products postprandial glucose (PPG) response as the main outcome and also insulin and incretin responses as secondary outcomes in patients with T2DM. Ten trials met the eligibility criteria. A random-effects model was used to obtain pooled effect size. **Results**: The pooled analysis of the included trials indicated a significant reduction in the mean of glucose in the area under the curve [standard mean difference (SMD)=-1.295, 95% CI: -1.878 to -0.712, P<0.001] and also an increase in the mean of insulin, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (SMD=0.562, 95% CI: 0.303 to 0.822, P<0.001, SMD=0.349, 95% CI: 0.074 to 0.624, P=0.013 and SMD=0.439, 95% CI: 0.154 to 0.724, P=0.003, respectively) in participants who consumed whey protein compared with the control group. **Conclusion**: According to the findings, whey protein intake is effective in reducing postprandial blood glucose as well as increasing postprandial insulin and incretin levels. Thus, whey protein can be considered a strategy to improve glycemic control in patients with T2DM.

Introduction

As a serious and long-term metabolic disorder, Type 2 Diabetes mellitus (T2DM), leads to hyperglycemia and dyslipidemia through insulin resistance and even inadequate insulin secretion (DeFronzo, 2009). Postprandial hyperglycemia is an important factor that contributes to impaired glycemic response and progression of T2DM (Woerle *et al.*, 2007). Since the acute effect of

This paper should be cited as: Salimi Z, Asadi M, Mansoori A. ***The Effect of Whey Protein Consumption on Postprandial Glucose, Insulin and Incretin Responses in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis of Acute-Term Controlled Clinical Trials.*** *Journal of Nutrition and Food Security (JNFS)*, 2025; 10 (1): 178-193.

impaired glycemic response is notable in the metabolic and hormonal milieu, research on the postprandial period is an attraction for researchers. The postprandial hyperglycemia is related to an elevated risk of cardiovascular diseases and increased levels of hemoglobin A1C (HbA1c) in T2DM (Ceriello *et al.*, 2008, Rizza, 2010, Standl *et al.*, 2011). Thus, diet therapy should focus on reducing postprandial glucose (PPG) peaks during the day for patients with T2DM (Morgan *et al.*, 2012).

Dietary strategies, such as the consumption of complex carbohydrates or low-glycemic index diets, may contribute to improving metabolic health (Raben, 2014); however, it is difficult to keep up for a long time (Brekke *et al.*, 2004). According to the evidence, an adverse association between dairy intake and glycemia (Da Silva *et al.*, 2014, Drehmer *et al.*, 2015) as well as the occurrence of T2DM has been demonstrated (Aune *et al.*, 2013, Díaz-López *et al.*, 2016). Milk and other dairy products, which contain many bioactive compounds and essential nutrients, may play a major role in diminishing the risk of diseases (Lovegrove and Givens, 2016). Therefore, it is better to consider the effect of separated compounds in randomized controlled trials. It seems the intake of dairy proteins in particular whey protein has a protective role in metabolic consequences (Mignone *et al.*, 2015, Pal and Radavelli-Bagatini, 2013, Zhang *et al.*, 2016). The bovine milk protein contains two different protein types, namely casein (about 80%) and whey (about 20%). However, compared to casein, whey is richer in branched-chain amino acids including isoleucine, leucine, and valine (Hall *et al.*, 2003), which may contribute to major metabolic outcomes. unlike casein, which is passed slowly out of the stomach, whey protein has quick gastric emptying and enters the small intestine, causing further increases in plasma amino acids due to its acidic solubility (Pal and Radavelli-Bagatini, 2013).

Several randomized controlled trials (RCTs) have been conducted regarding the effects of whey protein on PPG, insulin, and incretin responses with inconsistent findings (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, Goudarzi and Madadlou, 2013, Jakubowicz *et al.*, 2014, Jakubowicz *et al.*, 2017, King *et al.*, 2018, Tessari *et al.*, 2007, Wu *et al.*, 2016). Some studies have revealed significant reductions in PPG (Frid *et al.*, 2005, Jakubowicz *et al.*, 2017, King *et al.*, 2018, Watson *et al.*, 2019, Wu *et al.*, 2016), increases in plasma insulin (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, Jakubowicz *et al.*, 2014, Jakubowicz *et al.*, 2017, King *et al.*, 2018, Tessari *et al.*, 2007, Wu *et al.*, 2016), glucagon (Bjørnshave *et al.*, 2018, Tessari *et al.*, 2007), and incretin hormones (total and intact) (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, Jakubowicz *et al.*, 2014, Jakubowicz *et al.*, 2017, Tessari *et al.*, 2007, Wu *et al.*, 2016), while other studies have found no change in PPG (Tessari *et al.*, 2007), plasma insulin, glucagon, and incretin hormones (Frid *et al.*, 2005, King *et al.*, 2018). Because of the conflicting results and the lack of a meta-analysis on this topic to date, the authors decided to carry out a systematic review and meta-analysis on acute-term RCTs to assess the overall effects of the whey protein on PPG, insulin, and gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) responses in patients with T2DM.

Materials and Methods

The present systematic review was accomplished according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher *et al.*, 2009). The protocol for this systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (http://www.crd.york.ac.uk/PROSPERO; registration no.: CRD42018110161).

Search strategy

A systematic search was performed for relevant studies in five major databases PubMed, Scopus, Web of Science, Embase, and Cochrane Library, and manually reviewed lists of published studies and articles in English from 2000 up to 2023 using the following search terms and MeSH terms in titles and abstracts of human studies:

(hyperglycemia OR glucose intolerance OR diabetes OR diabetes mellitus) AND (whey OR milk OR dairy OR Whey Proteins)).

Eligibility criteria

Original studies were included in meta-analysis if they were randomized parallel or crossover acute-term trials that investigated the effect of all types of whey protein including isolated form (90-95% protein; contains less lactose and fat), concentrated form (About 70–80% protein; lactose and fat) or hydrolyzed form (variable protein, lactose and fat concentrations) on PPG, insulin and incretins in patients with T2DM. The incremental area under the curve (IAUC)/area under the curve (AUC) of serum glucose levels was considered primary and insulin, GIP ,and GLP-1 were considered secondary outcomes (Brouns *et al.*, 2005). The exclusion criteria were as follows: Non-clinical trial design, no measuring PPG, non-diabetic participants, studies without a control group, prescription of the mixture of whey protein with other nutrients, insufficient data on postprandial glucose, insulin, incretin levels, and study duration. Study selection was performed by two independent authors (Z Salimi, A Mansoori) in a two-step process. First, to identify the eligibility of all found studies, the authors observed the titles and the abstracts. In the second step, the full-text versions of eligible trials in the first screening were independently evaluated for inclusion criteria by Z Salimi and A Mansoori.

Data extraction

Two researchers (Z Salimi and A Mansoori) extracted data from the eligible studies separately. The extracted information was as follows: 1) first author's name; 2) year of publication; 3) region and country; 4) the number of participants in the intervention and control groups; 5) gender, body mass index, and age of study participants; 6) study characteristics (study design, randomization method, and blindness); 7) reported data of iAUC/AUC for serum level of glucose, insulin, and incretin in the intervention and placebo groups. Any disagreement was resolved by discussion among the authors.

Quality assessment

The quality of the studies was independently evaluated by two of the authors using instructions described in the Handbook of Cochrane for Systematic Reviews and meta-analysis of interventions (Higgins and Green, 2011). Evaluation of each study was performed using the following items: sufficiency of sequence generation, adequacy of allocations concealment, blinding of participants and personnel, selective outcome reporting, defective outcome data, and other possible biases. Cochrane Handbook recommendations indicated that "yes", "no" and "unclear" items respectively demonstrated low, high, and unknown risk of bias. The overall quality of individual trials was classified into three categories: good (low risk for more than two items), fair (low risk for two items), or weak (low risk for less than two items).

Data analysis

The endpoints were the mean difference and standard deviation of PPG, insulin, GIP, and GLP-1 in the intervention/control group. When the exact value of iAUC/AUC was not mentioned in eligible studies, the authors used reported data in the charts and tables to obtain iAUC/AUC (Brouns *et al.*, 2005). The iAUC of Glucose levels in three studies (Bjørnshave *et al.*, 2018, Jakubowicz *et al.*, 2017, Watson *et al.*, 2019), insulin levels in two studies (Bjørnshave *et al.*, 2018, Jakubowicz *et al.*, 2017), GIP levels in two studies (Bjørnshave *et al.*, 2018, King *et al.*, 2018), and GLP-1 levels in two studies (Bjørnshave *et al.*, 2018, King *et al.*, 2018) were calculated using reported data in the charts of eligible studies. Also, the AUC of Glucose levels in three studies (Frid *et al.*, 2005, Goudarzi and Madadlou, 2013, Wu *et al.*, 2016), insulin levels in four studies (Frid *et al.*, 2005, Goudarzi and Madadlou, 2013, King *et al.*, 2018, Wu *et al.*, 2016), GIP levels in two studies (Frid *et al.*, 2005, Wu *et al.*, 2016), and GLP-1 levels in two studies (Frid *et al.*, 2005, Wu *et al.*, 2016) were calculated using reported data in the tables of these studies. Unit conversion was done in two studies for glucose (mol/l and mg/dl into mmol/l)

(Jakubowicz *et al.*, 2013, Tessari *et al.*, 2007) and in four studies for insulin (nmol/l and pmol/l into mU/l) (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, Goudarzi and Madadlou, 2013, Jakubowicz *et al.*, 2014) and in three studies for GIP (pmol/l into pg/ml) (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, Tessari *et al.*, 2007).

The pooled estimation of the standard mean difference (SMD) between the whey protein supplementation group and the control group was calculated using a random effect model. Between-study heterogeneity was tested using the χ^2 test or the same Cochran's Q test and I^2 and subgroup analyses were also performed to detect sources of heterogeneity (Lau *et al.*, 1997). Sensitivity analysis was performed using the leave -one -study out (one-study removed) approach. Thus, the authors were able to explore the effect of each study on the overall effect size. For an investigation of possible publication bias, the visual funnel plot and Egger's test was used (Egger *et al.*, 1997).

Results

Literature search

The initial search yielded 1472 potentially relevant citations, to which 127 articles were added following manual search, which was done on initial studies. 392 duplicated articles were removed. Toweny one eligible studies were selected for full-text review based on title and abstract analysis. Eventually, after careful assessment, 10 articles were included in the present systematic review. Reasons for non-admission of the other articles were as follows: not being postprandial (Daly *et al.*, 2014, Flaim *et al.*, 2017, Gaffney *et al.*, 2018), having no control group (Mortensen *et al.*, 2012, Mortensen *et al.*, 2009), prescription of the mixture of whey protein with other nutrients (Ang *et al.*, 2012), participants without T2DM (Akhavan *et al.*, 2010), lack of clarity of blood glucose curve and iAUC/AUC measurement for glucose (King *et al.*, 2018), having unusable results (Almario *et al.*, 2017) and unclear study time (Jakubowicz *et al.*, 2016). The steps of the study selection process are illustrated in **Figure 1**.

Characteristics of included studies

Ten clinical trial studies included in the meta-analysis involved 271 participants. The largest trial had 22 participants and the smallest trial had 10 participants. Three trials used whey protein isolate (WPI) (Goudarzi and Madadlou, 2013, Tessari *et al.*, 2007, Wu *et al.*, 2016), two studies used whey protein hydrolysate (WPH) (Goudarzi and Madadlou, 2013, King *et al.*, 2018), and three studies used whey protein concentrate (WPC) (Jakubowicz *et al.*, 2013, Jakubowicz *et al.*, 2014, King *et al.*, 2018). Whey protein was consumed in the rest of the trials. The iAUC/AUC of Glucose, insulin, and incretin were assessed ranging from 180 min to 360 min. Ranges of whey protein dose were from 0.1 g/kg body weight to 50 g which was served with meal or pre-meal in the intervention group vs. meal without whey protein in the control group. The characteristics and results of the studies are given in **Table 1**.

Assessment of risk of bias

The quality of studies was assessed based on the domains of the Cochrane Collaboration's tool applied, including random sequence generation, allocation concealment, participants and personnel blinding, incomplete outcome data, selective reporting, and other sources of bias. The results were divided into three groups: high, low, and unclear risk of bias. The interpretation of the quality assessment results was as follows: fair (low risk for 2 items), weak (low risk for less than 2 items), or good (low risk for more than 2 items). Among 10 studies investigated in this systematic review, one was classified to have a good quality (Goudarzi and Madadlou, 2013), 3 were considered to have a fair quality (Bjørnshave *et al.*, 2018, Tessari *et al.*, 2007, Wu *et al.*, 2016), and 6 were of rather poor quality (Frid *et al.*, 2005, Jakubowicz *et al.*, 2013, Jakubowicz *et al.*, 2014, Jakubowicz *et al.*, 2017, King *et al.*, 2018, Watson *et al.*, 2019)

Findings from the meta-analysis

The consumption of whey protein compared with control demonstrated a significant reduction

in glucose AUC mean (SMD=-1.29, 95% CI: -1.87 to -0.71, *P*=0.001), and with significant heterogeneity (*P* for heterogeneity <0.001, I^2=85.7%, **Figure 2**) in the pooled analysis of 9 studies (15 comparisons). According to the Glucose Influence Analysis, the deletion of any of the studies did not change the result of the analysis and they were in a range from -1.40 (95% CI: -2 to -0.79) to -1.19 (95% CI:-1.77 t0 -0.60). The Pooled data of 9 studies (16 comparisons) that reported insulin showed a significant increase in insulin AUC mean in participants who consumed whey protein compared with control (SMD=0.562, 95% CI: 0.303 to 0.822, *P*<0.001) (*P* for heterogeneity=0.050, I^2=40.0%, **Figure 3**). Five studies (7 comparisons) described data on GIP AUC pooled data and showed a significant increase in GIP AUC mean in participants who consumed whey protein compared with control (SMD=0.34, 95% CI: 0.07 to 0.62, *P*=0.013) (*P* for heterogeneity= 0.81, I^2=0.0%, **Figure 4**). The pooled data of 5 studies (7 comparisons) that reported GLP-1 showed a significant increase in GLP-1 AUC mean in participants who consumed whey protein compared with control (SMD=0.43, 95% CI: 0.15 to 0.72, *P*=0.003) (*P* for heterogeneity=0.39, I^2=4.7%, **Figure 5**).

Discussion

The present systematic review and meta-analysis of acute-term RCTs suggested that whey protein consumption significantly decreased PPG and increased post-meal insulin and incretin levels.

The number of acute-term studies published on the effects of whey protein intake on postprandial glycemic responses has increased in recent years. Several human studies have reported that whey protein intake significantly reduced PPG and increased postprandial insulin after food intake (Goudarzi and Madadlou, 2013, Jakubowicz *et al.*, 2013, Jakubowicz *et al.*, 2014, Jakubowicz *et al.*, 2017, King *et al.*, 2018, Wu *et al.*, 2016). Nevertheless, according to the results of the study conducted by Tessari *et al*, following the ingestion of whey protein and free amino acid and casein amino acids, postprandial insulinemia was higher during whey protein consumption (Tessari *et al.*, 2007). Inversely, PPG levels were lower during free amino acid intake compared with whey protein. Besides, the findings of Frid (Frid *et al.*, 2005) suggested that replacing lean ham and lactose with an equal amount (18·2 g) of whey protein in high GI meals, significantly increased the insulin response but did not have any significant effects on glucose response following the breakfast meal. Furthermore, studies accomplished by Almario *et al.* and Bjørnshave *et al* showed similar results in PPG and insulin response (Almario *et al.*, 2017, Bjørnshave *et al.*, 2018). It is possible that using a low dosage of whey protein because of insufficient increase in post-breakfast circulating insulin to overcome insulin resistance in the post-absorptive state, and also higher levels of insulin resistance after the nocturnal fasting (Plat *et al.*, 1996), were the causes of less-reported PPG after whey protein intake in a breakfast meal.

Studies have also shown that taking whey protein increased GLP-1 (Almario *et al.*, 2017, Jakubowicz *et al.*, 2014, Jakubowicz *et al.*, 2017, Tessari *et al.*, 2007, Wu *et al.*, 2016) and GIP levels (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, Tessari *et al.*, 2007, Wu *et al.*, 2016). Interestingly, in some studies, whey protein intake did not have a significant effect on postprandial GLP-1 (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, King *et al.*, 2018) and GIP levels (King *et al.*, 2018). The reason for the discrepancy could be related to the different doses of whey protein. Studies showed that administration of larger doses of whey protein, from 25 to 50 g, significantly increased incretin response (Almario *et al.*, 2017, Jakubowicz *et al.*, 2014, Jakubowicz *et al.*, 2017, Tessari *et al.*, 2007, Wu *et al.*, 2016), whereas administration of smaller doses, from 15 g to 20 g, showed no difference in incretin responses (Bjørnshave *et al.*, 2018, Frid *et al.*, 2005, King *et al.*, 2018).

According to the available evidence, whey protein reduces blood glucose through insulin-dependent and insulin-independent mechanisms (Akhavan *et al.*, 2014). Although not fully determined, whey protein consumption seems to

either directly or indirectly increase postprandial insulin (Floyd *et al.*, 1966). Due to rapid digestion and high solubility of whey protein, plasma amino acids specifically leucine, isoleucine, and valine, rapidly increase (Pal and Radavelli-Bagatini, 2013) and directly induce the insulinotropic/β-cell-stimulating effects (Bosscher *et al.*, 2009). In addition, amino acids and bioactive peptides obtained from gastrointestinal digestion of whey protein stimulate L cell activity enhance their proliferation, and secrete GLP-1 and other incretin hormones (Jakubowicz and Froy, 2013). Also, whey protein may serve as an endogenous inhibitor of dipeptidyl peptidase-4 in the intestine, and as a result, prevent the local GLP-1 degradation after its release from the enteroendocrine cells (Jakubowicz and Froy, 2013, Power-Grant *et al.*, 2015). In addition, whey protein and its digested peptides and amino acids, increase glucose uptake by an enhancement in the Akt phosphorylation in the muscle cells, and finally, the GLUT4 transmission to the plasma membrane. Furthermore, Whey protein intake decreases PPG through an insulin-independent reduction in the speed of gastric emptying (Marathe *et al.*, 2013).

Nevertheless, Smedegaard *et al* investigated the effect of pre-meal iso-nitrogenous amounts of whey protein isolate and β-lactoglobulin (the main component of whey protein) in T2DM patients on postprandial level of insulin and glucagon that were increased after intake of β-lactoglobulin compared with the whey protein isolate (Smedegaard *et al.*, 2021). Some components of whey protein could stimulate glucagon secretion along with insulin secretion, which should be considered in future studies.

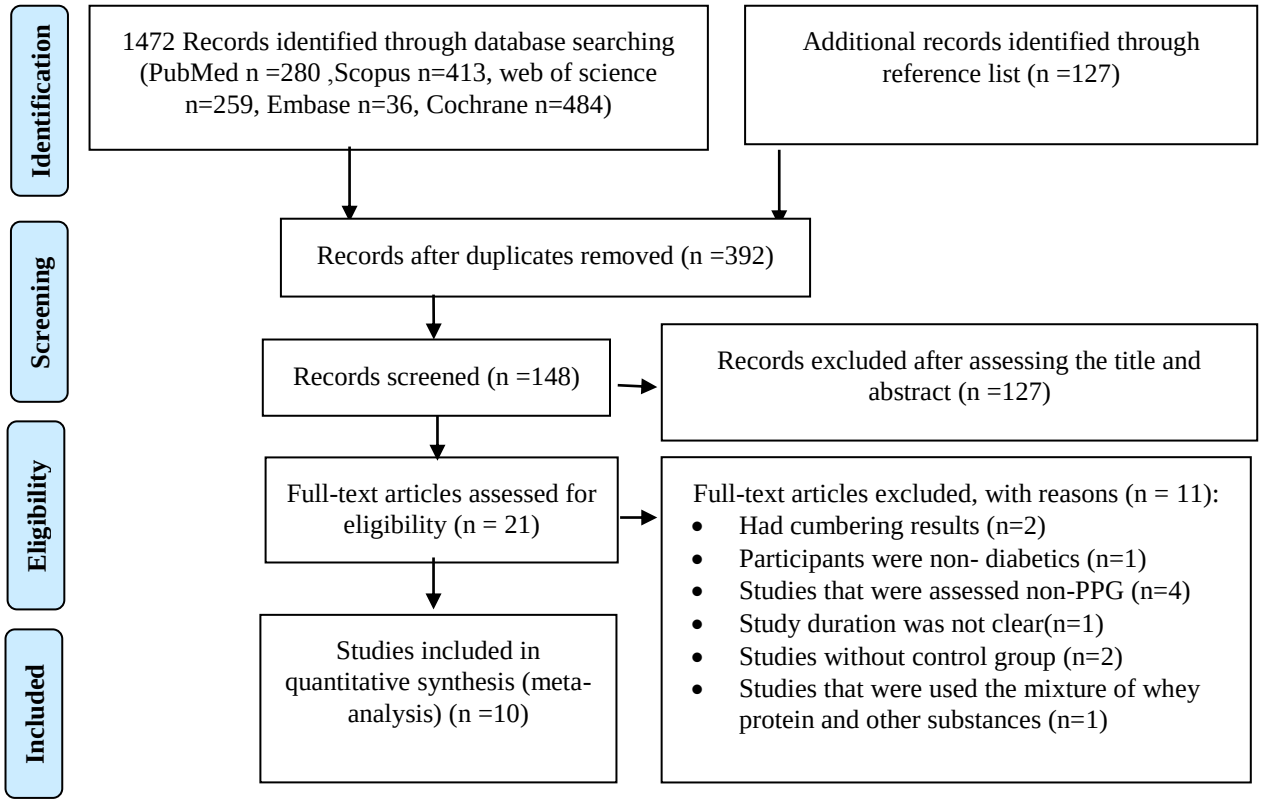

Figure 1. Flow diagram for selection of trials.

Table 1. Characteristics of trials included in the meta-analysis

Reference	Participants	No. of subjects (whey/ control)	Design study	Group characteristic		Postprandial test type glucose/insulin/GIP /GLP	PPG		Postprandial insulin		Postprandial GIP		Postprandial GLP	
				Inte	Cont		Inte	Cont	Inte	Cont	Inte	Cont	Inte	Cont
(Wu *et al.*, 2016)	type 2 diabetes Age: 64.2±1.4 years BMI: 27.9±1.7 kg/m^2	22/22	C	50 mg vildagliptin + 25 g whey isolate preload	50 mg vildagliptin +25 g control flavoring preload	AUC-30 to 240 min (mmol/l . h)/AUC-30 to 240 min (mU/l . h)/ AUC-30 to 240 min (pg/ml. h)/ AUC-30 to 240 min (pmol/l . h)	46.3	48.7	166.3	137.9	45.60	36.938	31.8	25.5
(Bjørnshave *et al.*, 2018)	type 2 diabetes Age: 62.9 years	12/12	C	20 g of WP (whey protein) dissolved in 200 mL water	200 ml water	iAUC-15 to 360 min (mmol/l)/iAUC-15 to 360 min (mU/l) / iAUC-15 to 360 min (pg/ml)/ iAUC-15 to 360 min (pmol/l)	272.73	360.13	6515.856	6062.4	4304.07	3890.54	7311	4844.5
(Frid *et al.*, 2005)	type 2 diabetes Age: 27–69 years BMI: 26.2±3.1kg/ m^2	14/14	C	18.2 g of whey powder dissolved in water	5.3 g lactose dissolved in water	iAUC 0-180 min (mmol · min/l)/ iAUC 0-180 min (mIU.min/l)/ iAUC0-180 min (pg/ml)/ iAUC0-180 min (pmol/l)	449	450	6379.2	5400	3122.85	2605.55	3088	2845
(Goudarzi and Madadlou, 2013)	type 2 diabetes Age: 32.4±4.1 years BMI: 26.2±1.2 kg/m^2	10/10	C	WPH (hydrolysate)beverages (0.1 g kg body weight)	Control (distilled water)	iAUC 0-180 min (mmol · min/l)/ iAUC 0-180 min (mIU.min/l)	462	559.68	3525.12	2371.5				

(Goudarzi and Madadlou, 2013)	type 2 diabetes Age: 32.4±4.1 years BMI: 26.2±1.2 kg/m^2	10/10	C	WPH beverages (0.2g kg body weight)	Control (distilled water)	iAUC 0-180 min (mmol · min/l)/ iAUC 0-180 min (mIU.min/l)	294.75	559.68	5692.464	2371.5
(Goudarzi and Madadlou, 2013)	type 2 diabetes Age: 32.4±4.1 years BMI: 26.2±1.2 kg/m^2	10/10	C	WPH beverages(0.4 g kg body weight)	Control (distilled water)	iAUC 0-180 min (mmol · min/l)/ iAUC 0-180 min (mIU.min/l)	213	559.68	9543.3552	2371.5
(Goudarzi and Madadlou, 2013)	type 2 diabetes Age: 32.4±4.1 years BMI: 26.2±1.2 kg/m^2	10/10	C	WPI(isolate) beverages (0.1, g kg body weight)	Control (distilled water)	iAUC 0-180 min (mmol · min/l)/ iAUC 0-180 min (mIU.min/l)	483	559.68	2111.97	2371.5
(Goudarzi and Madadlou, 2013)	type 2 diabetes Age: 32.4±4.1 years BMI: 26.2±1.2 kg/m^2	10/10	C	WPI beverages (0.2, g kg body weight)	Control (distilled water)	iAUC 0-180 min (mmol · min/l)/ iAUC 0-180 min (mIU.min/l)	469.5	559.68	3045.6	2371.5
(Goudarzi and Madadlou, 2013)	type 2 diabetes Age: 32.4±4.1 years BMI: 26.2±1.2 kg/m^2	10/10	C	WPI beverages (0.4 , g kg body weight)	Control (distilled water)	iAUC 0-180 min (mmol · min/l)/ iAUC 0-180 min (mIU.min/l)	297	559.68	4811.4	2371.5

(Jakubowicz *et al.*, 2013)	type 2 diabetes Age: 64±5.5 years BMI: 26.9±4.6 kg/m^2	15/15	C	50 g whey protein concentrate (WPC)dissolved in 250 ml water	250 ml water	AUC-15 to 180min (mmol/l)/AUC-15 to 180min (mU/ml)	41.38	56.99	367.9	178.95		
(Jakubowicz *et al.*, 2014)	type 2 diabetes	15/15	C	50 g whey protein concentrate dissolved in water	Water	iAUC 0-180 min (mmol/l)/ iAUC 0-180 min (mIU/l) / iAUC 0-180 min (pmol/l)	154	213.6	13263.84	6479.3		
(King *et al.*, 2018)	type 2 diabetes Age: 54.9±2.3 years BMI: 31.8±2.6 kg/m^2	11/11	C	15 g intact whey protein concentrate	Placebo beverage	iAUC 0-180 min (mIU /l))/ iAUC0-180 min (pg/ml)/ iAUC0-180 min (pmol/l)			15371.4	12779.	68727.22 58506.9	674.9 510.2
(King *et al.*, 2018)	type 2 diabetes Age: 54.9±2.3 years BMI: 31.8±2.6 kg/m^2	11/11	C	15 g hydrolyzed whey protein	Placebo beverage	iAUC 0-180 min (mIU /l)/ iAUC0-180 min (pg/ml)/ iAUC0-180 min (pmol/l)			14586.2	12779.	59005.065 58506.9	518.175 510.2
(Tessari *et al.*, 2007)	type 2 diabetes Age: 56.6±2.3 years BMI: 24.3±0.8 kg/m^2	12/12	C	Fast protein sweet whey protein isolate (WHEY)	Mixture of free L-amino acids	iAUC 0-180 min (mmol/l × 180 min)/ iAUC 0-180 min (nU/ml × 180 min))/ iAUC0-180 min (pg/ml. 180 min)/ iAUC-0-180 min (pmol/l .180 min)	2800	2700	33.2	35.3	1043.31 632.25	1254 1304

(Jakubowicz et al., 2017)	type 2 diabetes Age: 30–70 years BMI: 26–34 kg/m^2	17/16	P	(28 g) whey protein breakfast diet (WBdiet)	Protein breakfast diet (PBdiet)	iAUC 0-180 min (mmol/l)/ iAUC 0-180 min (mIU/ml*180 min)	288.9	447.75	8778.3	6607.2
(Mignone *et al.*, 2016)	type 2 diabetes	22/22	P	150 ml flavored shakes containing either 20 g whey protein and 5 g guar	Flavored placebo	iAUC -15-240 min(mmol · min/l)	4298.58	4665.6		

iAUC: Incremental area under the curve; AUC: Area under the curve; C: Cross-over; P: Parallel; Inte:Intervention; Cont: Control.

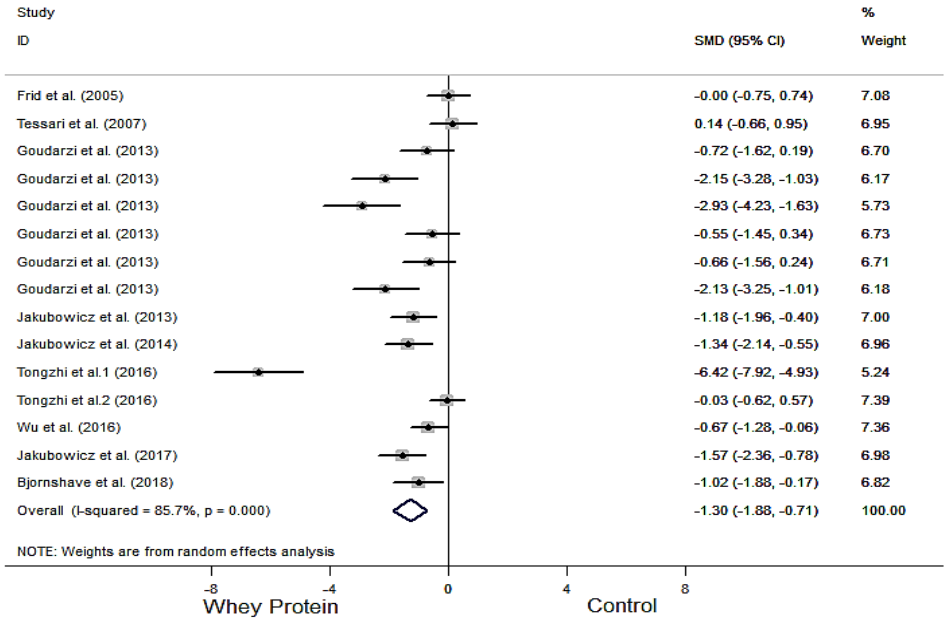

Figure2. Forest plot showing individual and pooled random effect standard mean difference (95% CI) of trials experimenting the whey protein effect on glucose area under the curve. Test of overall effect: z= 4.36, *P* = 0.001.

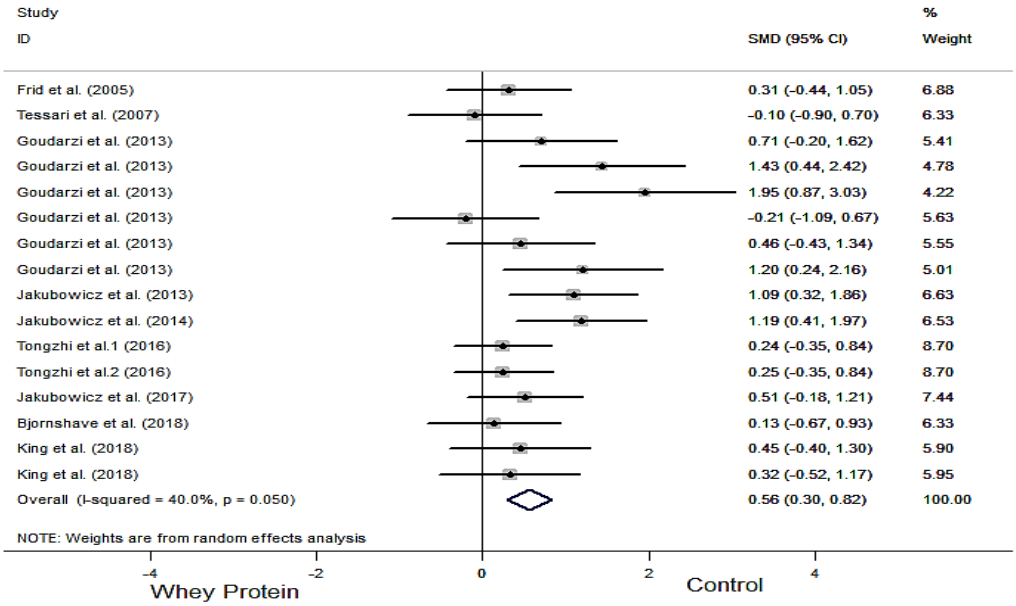

Figure3. Forest plot showing individual and pooled random effect regarding standard mean difference (95% CI) of trials experimenting the whey protein effect on insulin area under the curve. Test of overall effect: z= 4.25, *P* = 0.001.

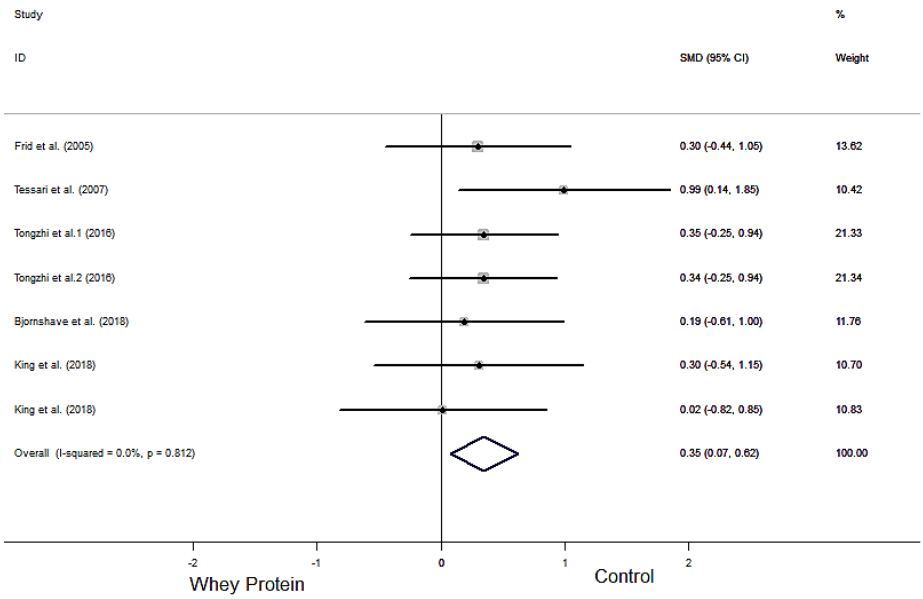

Figure4. Forest plot showing individual and pooled random effect regarding standard mean difference (95% CI) of trials experimenting the whey protein effect on GIP area under the curve. Test of overall effect: z= 2.49, *P* = 0.013.

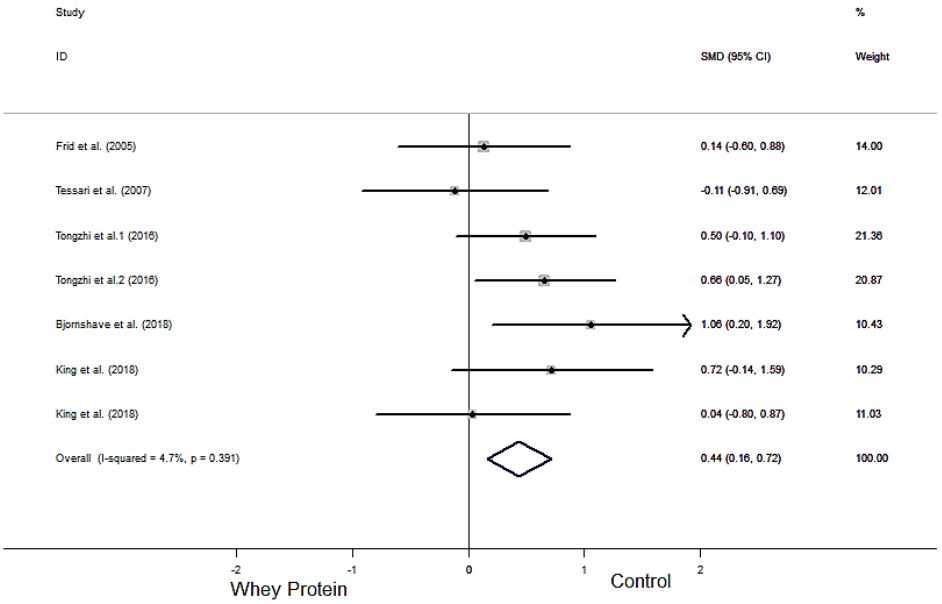

Figure5. Forest plot showing individual and pooled random effect regarding standard mean difference (95% CI) of trials experimenting the whey protein effect on GLP-1 area under the curve. Test of overall effect: z= 3.02, *P* = 0.003.

Although the present study was the first meta-analysis on this topic so far, there were some limitations. 1) AUC was calculated for glucose, insulin, and incretin levels by formulas in a few studies because their data were shown in the chart which may be considered an erroneous source. 2) There was language limitation for published trials. So, only English-published trials were included. 3) postprandial glycemic status was measured in different periods, although the authors used standardized methods for the elimination of the differences. 4) The authors could not perform

subgroup analysis based on dose and type of whey protein due to the small number of studies. 5) And most of the studies showed low quality because in such studies blinding and allocation concealment is difficult or sometimes unlikely. In the design of future studies, consideration of an efficacious dose to reduce postprandial hyperglycemia, avoiding overconsumption of energy, and ensuring palatability is recommended. In addition, since regular consumption of whey protein in high doses may hurt energy balance, the calorie of prescribed whey protein should be considered as a part of the total calorie.

Conclusion

In conclusion, the intake of whey protein exactly before or along with a meal reduced postprandial glucose and increased insulin, GIP, and GLP-1 in patients with T2DM. Since long-term treatment with a pre-meal low-dose whey/guar preload in patients with T2DM is associated with a clinically modest reduction in Hba1c, whey protein supplementation may be a new approach to improve glycemic control in patients with T2DM. However, long-term studies focusing on glycemic control are needed to achieve stronger results.

Acknowledgments

Nothing to declare

Authors' contributions

Z Salimi conceived the study and performed the literature search, data extraction, independent reviewing and carried out the quality evaluation of the included studies. A Mansoori conducted data analysis, interpretation and oversaw the methods. Z Salimi and M Asadi wrote the manuscript. All authors read and confirmed the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declared no conflict of interest.

Funding

This review did not receive any funding from the public and commercial agencies.

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