

# Whey protein preloading can alleviate stress adaptation disorder and improve hyperglycemia in women with gestational diabetes mellitus

**Yan Feng**

Qindao University Medical College Affiliated Yantai Yuhuangding Hospital <https://orcid.org/0000-0002-8884-8668>

**Qi Feng**

Chinese PLA General Hospital

**Xinna Song**

Qindao University Medical College Affiliated Yantai Yuhuangding Hospital

**Xiaoyan Li**

Qindao University Medical College Affiliated Yantai Yuhuangding Hospital

**Yinghong Zhang**

Qindao University Medical College Affiliated Yantai Yuhuangding Hospital

**Jianwei Hu** (✉ [yyfy190085@163.com](mailto:yyfy190085@163.com))

---

## Research

**Keywords:** Whey protein, Gestational diabetes mellitus, Oxidative stress, Insulin resistance, Stress adaptation

**Posted Date:** May 6th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-22728/v1>

**License:**   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at Gynecological Endocrinology on June 1st, 2021. See the published version at <https://doi.org/10.1080/09513590.2021.1932803>.

# Abstract

## Background

Whey protein (WP) intake has been shown to improve hyperglycemia.

## Objective

The aim of this study was to investigate the change of stress hormones, oxidative stress and insulin resistance (IR) in women with gestational diabetes mellitus (GDM) after supplement whey protein, in an attempt to gain insights into the prevention and treatment of GDM.

## Methods

60 GDM women were recruited in this study, and 30 women receive a preload drink containing 25 g whey protein as group GDM-W, and the other 30 women receive control flavoring drink as group GDM, and the trial last for 14 days. Plasma epinephrine (E), noradrenaline (NE), and cortisol were detected, we also investigate levels of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH). Homeostasis model assessment of insulin resistance (HOMA-IR) was used to assess IR.

## Results

In GDM-W group, postprandial blood glucose was decreased significantly on 3d, 5d, 7d and 14d ( $p < 0.05$ ), plasma 2 h insulin was increased by 7.2%, 8.6% and 20.5% on days 5, 7 and 14 ( $p < 0.05$ ). HOMA-IR was decreased significantly on days 14 ( $P < 0.01$ ). MDA was increased by 20.7% on days 14 ( $P < 0.01$ ), and anti-oxidative enzymes SOD was increased by 13.4% on days 14 ( $P < 0.01$ ) and GSH was increased by 16.7% and 29.1% on days 7 and 14 (both  $P < 0.05$ ). Stress hormones E and cortisol were decreased by 10.8% and 19.8% respectively on days 14 ( $p < 0.05$ ).

## Conclusions

Whey protein supplementation may improve hyperglycemia by alleviate stress disorder and oxidative stress injury in GDM women.

## Introduction

Gestational diabetes mellitus (GDM) is characterized by abnormal glucose tolerance during pregnancy, and it is a common complication of pregnancy, which can increase the risk of occurrence of type 2 diabetes mellitus (DM2) in both mothers and their offspring, also cause adverse effects on the outcome

of mothers and fetus[1, 2]. In recent years, the incidence of global GDM is on the rise. The incidence of GDM in pregnant women in China is as high as 22.94%, while the pathogenesis of GDM is not clear[3]. How to prevent and treat GDM and reduce the risks it brings?

Several studies show that Whey protein preloading can increase insulin sensitivity and have a positive response to the control of hyperglycemia[4, 5]. We previously reported that stress adaptation disorder exists in GDM women and may be associated with the pathogenesis of GDM[6, 7]. The purpose of our study is to explore the effect of whey protein supplementation on stress hormones in GDM women, and to observe the effect of whey protein preloading on postprandial blood glucose control in GDM patients, in an attempt to gain insights into the prevention and treatment of GDM.

## Materials And Methods

### Subjects

60 pregnant women patients with GDM at 24-28 weeks were included in this study.

According to the American Diabetes Association criteria, a 75-g oral glucose tolerance test (OGTT) at 24-28 weeks of pregnancy was used, GDM was diagnosed with the cutoff value being >5.1 mmol/L at fasting, >10.0 mmol/L at 1 h, and >8.5 mmol/L at 2 h[8]. They were divided into 2 groups consisting of 30 pregnant women each: GDM-W group receive 250ml preload drink containing 20 g whey protein isolate (73Kcal; Agropur inc, Le Sueur MN, America) 30 min before lunch and GDM group receive 250ml preload drink containing 20 g control flavoring (6.5 kcal; Cottee's, Southbank, Australia) 30 min before lunch. Blood samples were collected on days 3, 5, 7 and 14. The experiment lasts for 14 days.

Inclusion criteria were 1) single pregnancies; and 2) fasting plasma glucose (FPG) <6.1mmol/L before pregnancy. Exclusion criteria were 1) pregnant women with previously known medical complications during pregnancy such as DM 1 or 2, polycystic ovary syndrome, hyperthyreosis, hypothyroidism; and 2) women who were treated with hormones or drugs that may affect blood glucose.

The study protocol was approved by Yantai yuhuangding hospital committee, and informed consents of all participants were obtained.

### Characteristics of GDM women

Clinical features including maternal age, pre-gestational body mass index (BMI) and gestational weeks. Pre- BMI=pre-gestational weight (kg)/ height (m<sup>2</sup>).

### Blood glucose, stress hormones, oxidative stress injury markers and HOMA-IR in GDM women

Roche automatic biochemical analyzer (Roche Diagnostics, Mannheim, Germany) was used to detect blood glucose, and electrochemical luminescence immunoassay (Roche Diagnostics, Mannheim,

Germany) was used to test fasting and plasma 2h insulin levels. Insulin resistance (IR) was assessed by homeostatic model assessment of insulin resistance (HOMA-IR) formula[9].

Plasma epinephrine (E), noradrenaline (NE) and cortisol were used as markers of stress hormones. E and NE levels were determined by radioimmunoassay (RIA) (Roche Diagnostics, Mannheim, Germany). Electrochemical luminescence immunoassay (Roche Diagnostics, Mannheim, Germany) was used to detecte cortisol levels.

Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione (GSH) were used as markers of oxidative stress. MDA, SOD and GSH were detected by their correspondng assay kits (Jiancheng Bioengineering Institute, Nanjing, China). Results are expressed as umol/L, U/ml and mg/L.

## Statistical analysis

Continuous variables of normal distribution data are presented as mean  $\pm$  standard error. Independent-Samples t test was used to identify between-group differences. Data not normally distributed were log-transformed before analysis.  $P < 0.05$  was considered statistically significant. Statistical analyses were performed with SPSS 19.0 (SPSS, Inc., Chicago, IL, USA).

## Results

### Maternal characteristics

The maternal characteristics including the maternal age, pre-gestational BMI and gestational weeks were similar in GDM and GDM-W groups at entry to the study, there was no significant difference between two groups ( $p > 0.05$ )(Table 1).

Table 1 Maternal characteristics in GDM and GDM-Whey preload women ( $x \pm s$ )

	GDM	GDM-W
Maternal age (years)	26.5 $\pm$ 2.3	25.9 $\pm$ 3.1
Pre-gestational BMI (Kg/m <sup>2</sup> )	23.5 $\pm$ 2.7	24.0 $\pm$ 3.4
Gestational weeks	26.4 $\pm$ 1.8	25.2 $\pm$ 2.4

### Effects of whey preload on blood glucose related indexes in GDM women

There was no significant difference in fasting blood glucose bewteen two groups during the entire experimental session ( $p > 0.05$ ) (Fig. 2A).

In GDM-W group, postprandial 2 h blood glucose was decreased significantly on 3d, 5d, 7d and 14d compared with GDM group ( $p < 0.05$ ) (Fig. 2B).

After whey-perload, plasma 2 h insulin was increased by 7.2%, 8.6% and 20.5% on days 5, 7 and 14 in GDM-W group ( $p < 0.05$ ) (Fig. 2C).

Whey-perload decreased HOMA-IR significantly on days 14 in the experimental session ( $P < 0.01$ ) (Fig. 2D).

### **Effects of whey preload on oxidative stress injury in GDM women**

The concentration of MDA in GDM-W group was increased by 20.7% on days 14, and showing significant difference between two groups ( $P < 0.01$ ) (Fig. 2E).

Anti-oxidative enzymes SOD in GDM-W group was increased by 13.4% on days 14 ( $P < 0.01$ ) (Fig. 2F), and same trend was observed in GSH, compared with GDM group, the content of GSH in GDM-W group was increased by 16.7% and 29.1% on days 7 and 14 (both  $P < 0.05$ ) (Fig. 2G).

### **Effects of whey preload on plasma stress hormones in GDM women**

Compared with GDM group, stress hormones E and cortisol were decreased by 10.8% and 19.8% respectively on days 14 ( $p < 0.05$ ) (Fig. 2H, 2J). However no significant difference between the two groups in NE ( $p > 0.05$ ) (Fig. 2I).

## **Discussion**

Our study showed that whey protein preload can reduce hyperglycemia during 14 days exposure in GDM women. Compared to GDM group, there was no significant difference in fasting blood glucose between two groups, but postprandial blood glucose was decreased significantly during the entire experimental session. The results are consistent with previous studies that used whey protein preload[5, 10–12], but the mechanism is different. Some studies suggest that the hyperglycemia-reducing effect of whey protein preload may relate to insulin stimulation and delaying of glucose absorption in intestinal[13–15], and we have got similar result, plasma 2 h insulin was increased on days 5, 7 and 14 in GDM-W group, HOMA-IR decreased significantly on days 14 in the experimental session. Some studies found that slower gastric emptying may be responsible for hyperglycemia-reducing[12, 16, 17].

E, NE and cortisol are markers of stress hormones, especially cortisol. Cortisol could increase hepatic glucose production, decrease insulin secretion and aggravate  $\beta$  cell function, all of which could lead to hyperglycemia[18].

Pregnancy is a process of slight and chronic stress. Our previous study showed that GDM women exist stress adaptive disorder, which is related to hyperglycemia and IR[6]. This study found that stress hormones E and cortisol were decreased by 10.8% and 19.8% respectively on days 14 after whey protein preload. Several studies have also found that cortisol was reduced after whey protein supplementation, which is similar to our result[19, 20]. It indicates that whey protein supplementation can reduce the stress adaptation disorder of GDM patients.

Previous studies on the relation of oxidative stress damage and whey protein supplementation have different conclusions. Some studies believe that supplement whey protein can increase the levels of oxidative stress products [21, 22], while others showed whey protein can reduce oxidative damage [23–25]. During our experiment session, oxidative stress products MDA was increased by 20.7% on days 14, anti-oxidative enzymes SOD was increased by 13.4% on days 14, and GSH was increased by 16.7% and 29.1% on days 7 and 14 after whey preload. So our results suggest that oxidative stress injury was decreased after supplement of whey protein.

Our study indicates that, whey protein preloading improved hyperglycemia and blood glucose related indexes, which may be related to the increase of insulin secretion, the improvement of IR, the delay of stress adaptation disorder, and the reduce of oxidative stress injury in GDM women.

## Conclusions

Studies assessing effects of whey protein supplementation on diabetes are relatively few in number, and none of these studies focus on GDM. Only 60 GDM patients were included in our study, and one GDM patients quit because of protein flavour during the experiment. Our observations support the conclusion of whey protein preloads could improve stress adaptation disorder, oxidative stress injury and HOMA-IR, which should be explored with larger clinical trials in patients with GDM.

## Abbreviations

GDM, Gestational diabetes mellitus;

GDM-W, Gestational diabetes mellitus preload whey protein group;

OGTT, Oral glucose tolerance test;

FPG, Fasting plasma glucose;

BMI, Body mass index;

IR, Insulin resistance;

HOMA-IR, Homeostatic model assessment of insulin resistance;

E, Epinephrine;

NE, Noradrenaline;

RIA, Radioimmunoassay;

MDA, Malondialdehyde;

SOD, Superoxide dismutase;

GSH, Glutathione.

## Declarations

### Conflicts of interest

None of the authors has a conflict of interest to declare.

### Acknowledgements

We would like to thank Wenting Wang for his help with analyzing the data.

### Authors' contributions

The authors' responsibilities were as follows: YF, QF, XNS, XYL, YHZ, JWH

YF and JWH: designed the study and wrote the manuscript; QF: performed the data analysis; XNS, XYL, YHZ, provided study oversight; all authors: approved the final manuscript. The authors report no conflicts of interest.

### Funding

This work was supported by grants from the Yantai science and technology innovation development plan project (2020YD028).

### Ethics approval

Approval was obtained from Ethics committee of Yantai yuhuangding Hospital [approval NO. 2018-104].

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing interests.

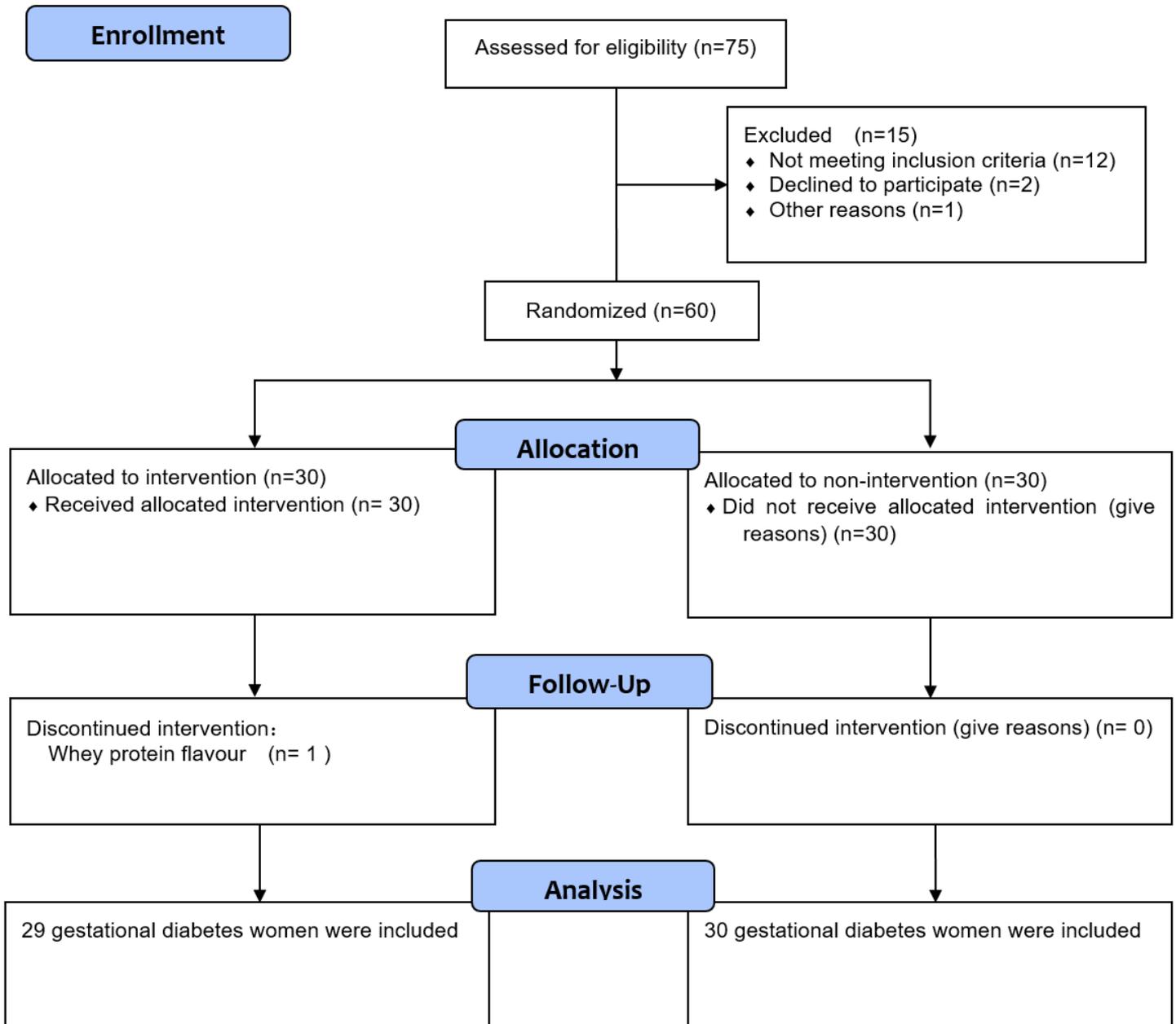
## References

1. Hirsch L, Berger H, Okby R, Ray JG, Geary M, McDonald SD, Murray-Davis B, Riddell C, Halperin I, Hasan H, et al: **Gestational diabetes mellitus is associated with adverse outcomes in twin pregnancies.** *Am J Obstet Gynecol* 2019, **220**:102.e101-102.e108.

2. Sesmilo G, Prats P, Garcia S, Rodríguez I, Rodríguez-Melcón A, Berges I, Serra B. **First-trimester fasting glycemia as a predictor of gestational diabetes (GDM) and adverse pregnancy outcomes.** *Acta Diabetol* 2020.
3. He Z, Xie H, Liang S, Tang Y, Ding W, Wu Y, Ming WK. Influence of different diagnostic criteria on gestational diabetes mellitus incidence and medical expenditures in China. *J Diabetes Investig.* 2019;10:1347–57.
4. D'Souza K, Mercer A, Mawhinney H, Pulinilkunnil T, Udenigwe CC, Kienesberger PC. Whey Peptides Stimulate Differentiation and Lipid Metabolism in Adipocytes and Ameliorate Lipotoxicity-Induced Insulin Resistance in Muscle Cells. *Nutrients.* 2020;12:1–18.
5. Zarkos J, Addai D, Tolekova A. Low Protein Diets for Pregnant Women and Its Association with Insulin Secretion and Resistance. *Open Access Maced J Med Sci.* 2019;7:686–9.
6. Feng Y, Feng Q, Yin S, Xu X, Song X, Qu H, Hu J. Stress adaptation disorders play a role in rat gestational diabetes with oxidative stress and glucose transporter-4 expression. *Gynecol Endocrinol.* 2020;1:1–5.
7. Feng Y, Feng Q, Qu H, Song X, Hu J, Xu X, Zhang L, Yin S. Stress adaptation is associated with insulin resistance in women with gestational diabetes mellitus. *Nutr Diabetes.* 2020;10:4.
8. Wei Y, Yang H, Zhu W, Yang H, Li H, Yan J, Zhang C. International Association of Diabetes and Pregnancy Study Group criteria is suitable for gestational diabetes mellitus diagnosis: further evidence from China. *Chin Med J (Engl).* 2014;127:3553–6.
9. Abbasi F, Okeke Q, Reaven GM. Evaluation of fasting plasma insulin concentration as an estimate of insulin action in nondiabetic individuals: comparison with the homeostasis model assessment of insulin resistance (HOMA-IR). *Acta Diabetol.* 2014;51:193–7.
10. Pham H, Holen IS, Phillips LK, Hatzinikolas S, Huynh LQ, Wu T, Hausken T, Rayner CK, Horowitz M, Jones KL. The Effects of a Whey Protein and Guar Gum-Containing Preload on Gastric Emptying, Glycaemia, Small Intestinal Absorption and Blood Pressure in Healthy Older Subjects. *Nutrients.* 2019;11:2666.
11. Watson LE, Phillips LK, Wu T, Bound MJ, Checklin H, Grivell J, Jones KL, Horowitz M, Rayner CK. Title: Differentiating the effects of whey protein and guar gum preloads on postprandial glycemia in type 2 diabetes. *Clin Nutr.* 2019;38:2827–32.
12. Bjornshave A, Holst JJ, Hermansen K. A pre-meal of whey proteins induces differential effects on glucose and lipid metabolism in subjects with the metabolic syndrome: a randomised cross-over trial. *Eur J Nutr.* 2019;58:755–64.
13. Stevenson EJ, Allerton DM. The role of whey protein in postprandial glycaemic control. *Proc Nutr Soc.* 2018;77:42–51.
14. King DG, Walker M, Campbell MD, Breen L, Stevenson EJ, West DJ. A small dose of whey protein co-ingested with mixed-macronutrient breakfast and lunch meals improves postprandial glycemia and suppresses appetite in men with type 2 diabetes: a randomized controlled trial. *Am J Clin Nutr.* 2018;107:550–7.

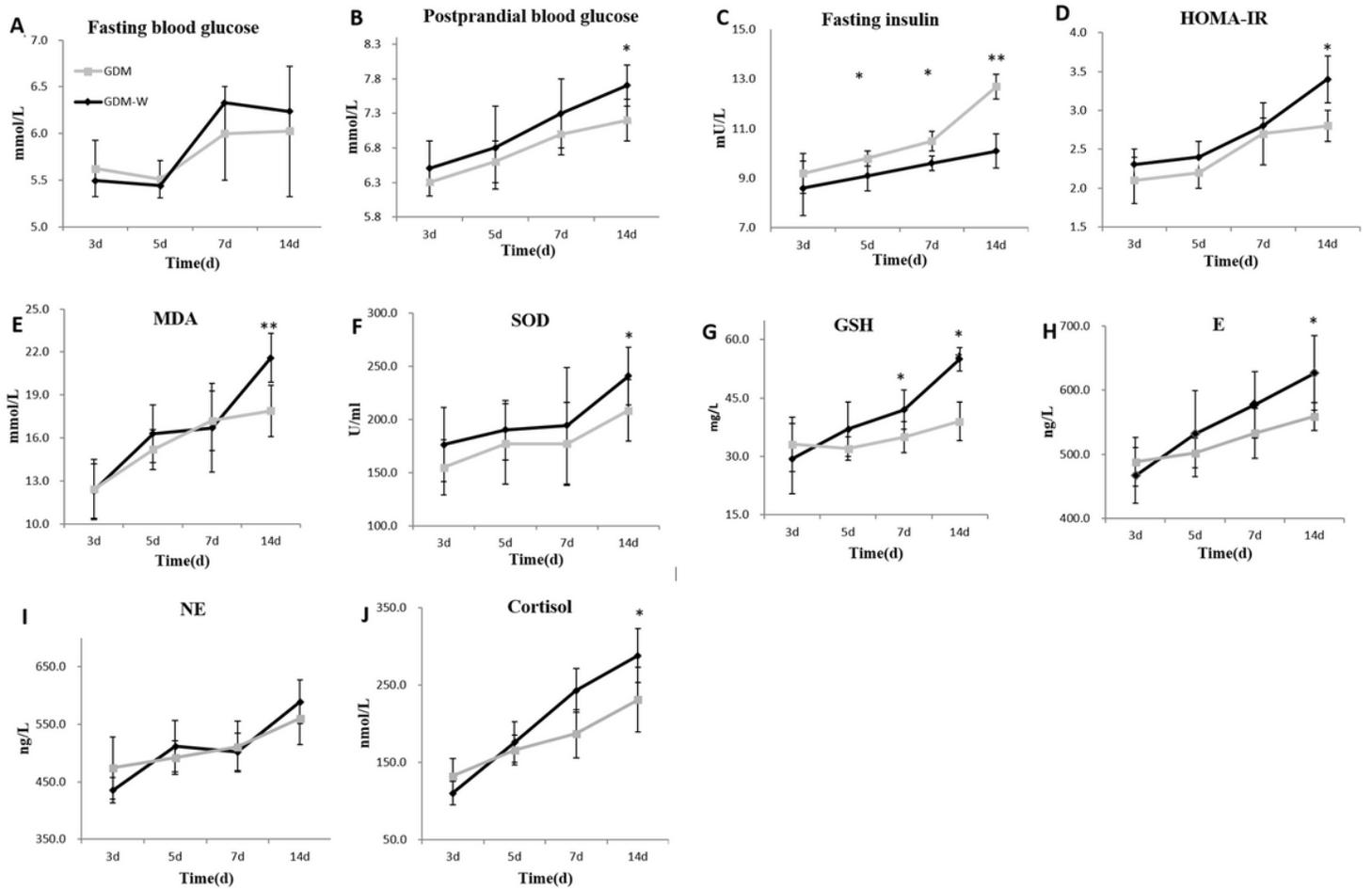
15. Bae JH, Kim LK, Min SH, Ahn CH, Cho YM. Postprandial glucose-lowering effect of premeal consumption of protein-enriched, dietary fiber-fortified bar in individuals with type 2 diabetes mellitus or normal glucose tolerance. *J Diabetes Investig.* 2018;9:1110–8.
16. Kung B, Anderson GH, Parodi S, Tucker AJ, Vien S, Wright AJ, Goff HD. Effect of milk protein intake and casein-to-whey ratio in breakfast meals on postprandial glucose, satiety ratings, and subsequent meal intake. *J Dairy Sci.* 2018;101:8688–701.
17. Giezenaar C, Luscombe-Marsh ND, Hutchison AT, Lange K, Hausken T, Jones KL, Horowitz M, Chapman I, Soenen S. Effect of gender on the acute effects of whey protein ingestion on energy intake, appetite, gastric emptying and gut hormone responses in healthy young adults. *Nutr Diabetes.* 2018;8:40.
18. Ortiz R, Kluwe B, Odeh JB, Echouffo Tcheguie JB, Sims M, Kalyani RR, Bertoni AG, Golden SH, Joseph JJ. The association of morning serum cortisol with glucose metabolism and diabetes: The Jackson Heart Study. *Psychoneuroendocrinology.* 2019;103:25–32.
19. Martin-Rincon M, Perez-Suarez I, Pérez-López A, Ponce-González J, Morales-Alamo D, de Pablos-Velasco P, Holmberg H, Calbet J. Protein synthesis signaling in skeletal muscle is refractory to whey protein ingestion during a severe energy deficit evoked by prolonged exercise and caloric restriction. *Int J Obes (Lond).* 2019;43:872–82.
20. Qin L, Wong SHS, Sun FH, Huang Y, Sheridan S, Sit CHP. Effects of Alpha-Lactalbumin or Whey Protein Isolate on Muscle Damage, Muscle Pain, and Mood States Following Prolonged Strenuous Endurance Exercise. *Front Physiol.* 2017;8:754.
21. Aydin B, Atli Sekeroglu Z, Sekeroglu V. Effects of whey protein and conjugated linoleic acid on acrolein-induced cardiac oxidative stress, mitochondrial dysfunction and dyslipidemia in rats. *Biomed Pharmacother.* 2018;107:901–7.
22. Zebrowska-Gamdzyk M, Maciejczyk M, Zalewska A, Guzinska-U K, Tokajuk A, Car H. Whey Protein Concentrate WPC-80 Intensifies Glycoconjugate Catabolism and Induces Oxidative Stress in the Liver of Rats. *Nutrients.* 2018;10:1178.
23. El-Desouky WI, Mahmoud AH, Abbas MM. Antioxidant potential and hypolipidemic effect of whey protein against gamma irradiation induced damages in rats. *Appl Radiat Isot.* 2017;129:103–7.
24. McDonald JD, Mah E, Chitchumroonchokchai C, Dey P, Labyk AN, Villamena FA, Volek JS, Bruno RS. Dairy milk proteins attenuate hyperglycemia-induced impairments in vascular endothelial function in adults with prediabetes by limiting increases in glycemia and oxidative stress that reduce nitric oxide bioavailability. *J Nutr Biochem.* 2019;63:165–76.
25. Corrochano AR, Ferraretto A, Arranz E, Stuknyt M, Bottani M, O'Connor PM, Kelly PM, De Noni I, Buckin V, Giblin L. Bovine whey peptides transit the intestinal barrier to reduce oxidative stress in muscle cells. *Food Chem.* 2019;288:306–14.

## Figures



**Figure 1**

Flowchart of enrollment and allocation of Whey protein preloading on GDM women



**Figure 2**

Fasting blood glucose (A), 2h blood glucose (B), 2h insulin (C), HOMA-IR (D), MDA (E), SOD (F), GSH (G), E (H), NE (I) and Cortisol (J) in response to whey preload 30 min before meal on 3d, 5d, 7d and 14d in patients with GDM.