Effect of oral protein hydrolysate on glucose control in patients with gestational diabetes

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Summary

Background & aims: In type 2 diabetic patients, a casein-based protein hydrolysate has been shown to increase plasma insulin and to lower plasma glucose. In the present study, we examined the acute and prolonged effects of protein hydrolysate on postprandial glucose, insulin and C-peptide responses after a standardised breakfast and the effect on daily glucose control in patients with gestational diabetes.

Methods: In a single-centre randomised double blind placebo controlled design, patients with mild gestational diabetes (no use of insulin or oral antidiabetic agents; n = 26/group) were allocated to receive a protein hydrolysate drink, 8.5 g before breakfast and 8.5 g before dinner or a placebo drink which was identical to the protein hydrolysate drink in appearance and taste, yet lacked carbohydrate, fat or protein, for 8 days.

Results: Baseline characteristics including fasting levels of glucose, insulin, C-peptide and insulin–glucose ratio were similar between the groups. Compared to the placebo drink, neither the first dose of the protein hydrolysate drink nor the final dose had effects on 4-h area under the curve for plasma levels of insulin and C-peptide, or the insulin-to-glucose ratio; however, plasma glucose was moderately lower between t = 45, 60 and 75 min. In addition, mean daily capillary glucose levels were lower in the protein hydrolysate group. Two patients in the PH drink group had to be withdrawn because of vomiting after the first dose.

Conclusions: In patients with gestational diabetes, a twice-daily dose of 8.5 g of protein hydrolysate of casein had no insulinotropic effects, but did moderately reduce plasma glucose levels, suggesting an increase in insulin sensitivity.

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1. Introduction

Gestational diabetes mellitus (GDM) occurs in about 5% of pregnancies. During pregnancy, insulin sensitivity declines with advancing gestation by about 50% due to high levels of several diabetogenic hormones [1]. Consequently, insulin secretion during pregnancy has to rise by a factor 2 to 2.5 to maintain a euglycaemic state. When insulin production and insulin sensitivity are mismatched, GDM evolves, a condition which is associated with adverse pregnancy outcomes, including macrosomia and related complications during labour, as well as an increased likelihood of developing diabetes in later life [2,3].

Dietary counselling, increased physical activity and self-monitoring of plasma glucose levels are recommended primary interventions for GDM [4]. In addition, pharmacological therapy such as insulin, metformin and glyburide are often required to maintain optimal glycaemic control. The disadvantages of insulin treatment include the risk of maternal hypoglycaemia, excessive weight gain and the necessity of multiple daily injections [5]. Conversely, metformin therapy causes adverse gastrointestinal effects as well as vitamin B12 deficiency and lactate acidosis [6], while the most common side effect of glyburide is hypoglycaemia [7]. These adverse side effects have prompted a search for alternative therapies to achieve glycaemic control in patients with GDM.
Positive correlations have been observed between the ingestion of protein and insulin secretion in non-pregnant type 2 diabetic patients [8–11]. Particularly, the ingestion of proteins rich in essential amino acids or fast-absorbable proteins has resulted in a greater postprandial insulin secretion than the ingestion of a ‘slow’ protein [11]. Furthermore, co-ingestion of protein hydrolysate (PH) in addition to carbohydrates has been shown to stimulate postprandial insulin secretion and to reduce postprandial plasma glucose increments in healthy volunteers and in patients with type 2 diabetes [12–14]. Based on these findings, we hypothesised that daily ingestion of PH in addition to following dietary recommendations improves glycaemic control in patients with GDM. To investigate this, plasma glucose, insulin and C-peptide concentrations were measured in patients with GDM randomised to either a twice-daily PH or placebo drink for 8 days to assess the acute and chronic effects.

2. Methods

2.1. Study participants

Participants were recruited between January 2012 to June 2015 from the Outpatient Clinic of the Erasmus Medical Centre (MC). Patients with GDM with a gestational age of ≥20+0 to 35+6 weeks were eligible to participate.

2.2. Diagnosis

Diagnosis of GDM was based on a plasma glucose concentration of ≥7.8 mmol/L 2 h post a 75-g oral glucose tolerance test (OGTT). Exclusion criteria for the study included treatment with insulin or oral antidiabetic agents, 3 plasma glucose concentration measurements of >9 mmol/L or 1 plasma glucose concentration measurement of >11 mmol/L after which subjects will start insulin treatment, pre-existing type 1 or 2 diabetes, serum alanine aminotransferase (ALAT) > 70 IU/L, renal insufficiency (eGFR <60 ml/min/1.73 m²) or clinical conditions or laboratory test results that could jeopardise the health status of the participants.

2.3. Study protocol

This study was a single-centre randomised double blind placebo controlled design and was approved by the Medical Ethical Committee of the Erasmus MC (MEC-2008-340) and registered in the Netherlands Trial Registry (NTR1848). After obtaining informed consent, participants were randomly assigned to the PH or placebo drink group using a block randomisation. Three days before the commencement of the study, participants received individualised dietary advice based on their nutritional requirements. On days 1 and 8 of the study, participants visited the Clinical Research Centre of the Erasmus MC. During these visits, a catheter was placed in an antecubital vein for blood sampling. After obtaining a fasting blood sample, participants consumed either 250 ml of the PH or placebo drink. Ingestion of the drink was designated as time = 0 min (t = 0). Immediately following t = 0, participants consumed a standardised breakfast (2 slices of white bread with marmalade and low-fat margarine) within a 15-min timeframe. The standardised breakfast consisted of 55 g carbohydrates, 5 g fat and 6 g protein, providing 291 kcal. Blood was drawn at t = 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210 and 240 min for the measurement of plasma glucose, insulin and C-peptide levels as described below. From days 1–7 of the study, participants consumed either 250 ml of the PH or placebo drink twice daily, one before breakfast and the second before dinner. On days 2–7, participants measured fasting capillary blood glucose levels 1.5 h after breakfast, lunch and dinner and just before bedtime. Patients were advised not to eat more than 175 g carbohydrates per day. Additionally, participants filled in a daily questionnaire to assess possible side effects that have been reported in previous publications concerning the use of PH drink. On day 8 of the study, participants visited the Clinical Research Centre of the Erasmus MC and the same 4-h protocol (as described above) for day 1 was followed. Thereafter the study was completed. In total, participants consumed the PH or placebo drink 15 times. Any unused and/or empty packages of the investigational product were returned at the completion of the study.

2.4. Investigational product

The PH was obtained by enzymatic hydrolysis of sodium caseinate (degree of hydrolysis 26%). The product was provided by DSM Food Specialties, Delft, The Netherlands. The 250 ml PH drink contained 8.5 g of protein hydrolysate providing 34 kcal. The placebo drink was identical in appearance and taste to the PH drink, yet lacked carbohydrates, fat and protein. The ready-to-drink PH and placebo formulations were produced and packaged at food-grade packaging facilities (Tetra Pak, Lund, Sweden).

2.5. Measurements

Measurement of plasma glucose, insulin and C-peptide levels were performed at the Clinical Laboratory of the Erasmus MC. Plasma glucose concentration was measured using the enzymatic hexokinase method (Roche/Hitachi cobas c systems, Manheim, Germany). Plasma insulin and C-peptide concentrations were measured using sandwich chemoluminescence immunoassays (Bad Nauheim, Germany) on Immulite 2000 XPi platforms.

2.6. Endpoints

The primary endpoint was the 4-h postprandial area under the curve (AUC) for plasma glucose, insulin and C-peptide levels and the insulin-to-glucose ratio on day 1 of the study, in line with previous studies showing an acute effect of PH on plasma insulin levels.

Secondary endpoints were 1) the AUCs for plasma glucose, insulin and C-peptide levels and the insulin-to-glucose ratio on day 8 of the study and 2) the fasting mean postprandial (average of 3 values) and mean daily capillary glucose concentration (average of 5 values) during days 2–7 of the study. The Homeostasis Model Assessment (HOMA) was used to quantify insulin resistance (IR) using the fasting glucose and insulin levels on days 1 and 8 of the study. The HOMA-IR was calculated with an installed HOMA2 calculator v2.2.3, using the formula HOMA-IR = insulin (mU/L) × glucose (mmol/L)/22.5.

2.7. Statistical analysis

For each participant, plasma glucose, insulin and C-peptide levels and the insulin-to-glucose ratio from t = 15 to t = 240 on days 1 and 8 of the study were calculated as AUCs above the baseline value obtained at t = 0. In addition, fasting, and average postprandial and average daily capillary glucose concentrations were calculated. Data are presented as median ± range or as mean values ± SEM or SD. For comparisons within and between groups, Student’s paired and unpaired t-tests and a two-way repeated measures ANOVA was used. For the entire group of participants, Pearson’s correlation coefficient was calculated to assess associations between continuous variables. A two-sided P-value < 0.05 was considered to indicate a significant difference. All statistical analyses were calculated with IBM SPSS Statistics 21 (IBM).
language barrier, one forgot to drink the drinks. Another three patients had increased plasma glucose concentration, three due to being too busy, work. Patients who did not meet the inclusion criteria or met the exclusion criteria for the study were excluded, three patients because of an increased plasma glucose concentration and one forgot to drink the drinks. Another two patients dropped out because of vomiting (Fig. 1). Twenty-four (48%) patients allocated to the PH group and 26 (52%) to the placebo group were available for analysis. At study entry, the groups did not differ in baseline characteristics or laboratory measurements (Table 1). During days 1 and 8 of the study, fasting plasma glucose concentrations on days 2–7 of the study.

### 3.2. 4-h postprandial area under the curve (AUC) of glucose, insulin, C-peptide and insulin-to-glucose ratio

The time courses of the responses of plasma glucose, insulin, C-peptide and insulin–glucose ratio during days 1 and 8 are displayed in Fig. 2. Although the AUCs for the 4 parameters did not differ, the 2-way repeated measures ANOVA showed that plasma glucose concentration in the PH group was lower than in the placebo group at t = 45–75 on day 1 of the study and at t = 45–60 on day 8 of the study. Within each group, the AUCs on day 1 and day 8 for the different parameters were closely correlated, with correlation coefficients ranging from 0.96 to 0.99 (P < 0.05).

### 3.3. Fasting, postprandial and daily capillary glucose concentrations on days 2–7 of the study

As displayed in Fig. 3, fasting glucose concentration was lower in the PH group than the placebo group on day 4 of the study, but not on any other day. Also, mean day glucose concentration (average of 5 values) was 5–10% lower on all days in the PH group when compared to the placebo group (P < 0.05), but no between-group difference in mean postprandial glucose concentration was detected.

### 3.4. Tolerability

As listed in Table 3, gastrointestinal side effects were similar between the two groups for participants who finished the study.

### 4. Discussion

The PH drink used in the present study has previously been shown to increase plasma insulin levels in non-pregnant healthy volunteers as well as in patients with type 2 diabetes mellitus when ingested with carbohydrates [13,15]. In a randomised controlled trial, we explored the acute and prolonged insulinotropic effects of a PH drink in patients with GDM. The PH drink in combination with a standard breakfast did not increase plasma insulin and C-peptide levels or the insulin to glucose ratio either acutely or after administration for 8 days. However, for limited time periods, the PH drink reduced plasma glucose concentration in GDM patients (Fig. 2). In addition, the average daily plasma glucose concentration was 5–10% lower in the PH group as compared to the control group.

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**Table 1**

<table>
<thead>
<tr>
<th>Patient characteristics on screening day.</th>
<th>PH</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Maternal age, years</td>
<td>30 ± 12</td>
<td>31 ± 8</td>
<td>0.836</td>
</tr>
<tr>
<td>GA at delivery, weeks</td>
<td>39 ± 1</td>
<td>38 ± 1</td>
<td>0.725</td>
</tr>
<tr>
<td>Maternal body weight in 1st trimester, kg</td>
<td>74 (44–140)</td>
<td>69 (45–127)</td>
<td>0.678</td>
</tr>
<tr>
<td>BMI in 1st trimester, kg/m²</td>
<td>26.7 (16.99–37.6)</td>
<td>27.7 (18.3–46.2)</td>
<td>0.46</td>
</tr>
<tr>
<td>GA at inclusion, weeks</td>
<td>29 ± 4</td>
<td>28 ± 4</td>
<td>0.412</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Fasting</td>
<td>4.9 (3.9–6.2)</td>
<td>5.1 (4.1–6.7)</td>
<td>0.451</td>
</tr>
<tr>
<td>- 2 h after OGTT</td>
<td>9.0 (7.8–12.3)</td>
<td>9.3 (7.9–12.1)</td>
<td>0.825</td>
</tr>
<tr>
<td>Serum ALAT, U/L</td>
<td>12 (5–23)</td>
<td>13 (5–30)</td>
<td>0.508</td>
</tr>
<tr>
<td>Serum creatinine, mmol/L</td>
<td>44 (28–61)</td>
<td>48 (30–75)</td>
<td>0.067</td>
</tr>
<tr>
<td>Haemoglobin, mmol/L</td>
<td>7.1 (5.8–8.4)</td>
<td>6.9 (5.7–8.0)</td>
<td>0.553</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median ± range; PH: Protein hydrolysate; GA: Gestational Age; BMI: Body Mass Index; OGTT: oral glucose tolerance test. ALAT: amino alanine transferase.
Several studies performed in healthy volunteers and in type 2 diabetic patients have shown that co-ingestion of a PH drink with carbohydrates is associated with an insulinotropic effect [13-16]. A PH dose of 0.175 g/kg administered for 165 min (about 41 g of PH in total per volunteer) was associated with an increase in the 4-h AUC of plasma insulin concentration of 132% in healthy controls and by 299% in type 2 diabetic patients as compared to carbohydrate ingestion alone [15]. In another study, the ingestion of a single drink of PH, equivalent to 0.3 g/kg body weight (approximately 25 g of PH per volunteer) together with carbohydrates resulted in a 66% greater 4-h AUC insulin response in healthy volunteers and a 141% greater response in type 2 diabetic patients as compared to carbohydrate ingestion alone. This insulinotropic response was associated with a 22 ± 32% and a 23 ± 36% decrease in plasma glucose concentration in healthy volunteers and type 2 diabetic patients respectively [16]. In another study in type 2 diabetic patients, co-ingestion of 6 g PH with 50 g carbohydrate had no effect on plasma insulin and glucose levels, whereas plasma insulin was higher and plasma glucose concentration lower with a dose of 12 g of PH [17]. These findings strongly suggest that to achieve an insulinotropic effect a threshold dose of PH is required. In the present study, we purposely selected a relatively low dose of PH (8.5 g) taken twice daily because of concerns of gastrointestinal tolerability of the drink in pregnant patients. In retrospect, we cannot exclude the possibility that the dose of PH used in the present study was too low to elicit an insulinotropic effect. In addition, the already high fasting insulin levels in our patients might have contributed to the lack of insulinotropic effect of the PH drink.

Although plasma insulin levels did not increase in response to PH, plasma glucose levels were 5—10% lower in the group allocated to the PH drink. An increase in insulin sensitivity induced by the ingestion of the PH drink is the most likely explanation for this observation. For instance, whey protein, which has a comparable amino acid content to the PH drink used in the present study, has been shown to reduce insulin resistance post-operatively in non-diabetic patients [18].

4.1. Strengths and limitations

To the best of our knowledge, this is the first study to determine both the short-term and long-term effects of PH on plasma insulin

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>PH Day 1</th>
<th>Pla Day 1</th>
<th>P</th>
<th>PH Day 8</th>
<th>Pla Day 8</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.8 ± 0.14</td>
<td>4.8 ± 0.12</td>
<td>0.984</td>
<td>4.7 ± 0.14</td>
<td>4.8 ± 0.11</td>
<td>0.567</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>109 ± 26</td>
<td>109 ± 28</td>
<td>0.600</td>
<td>96 ± 22</td>
<td>108 ± 28</td>
<td>0.861</td>
</tr>
<tr>
<td>C-peptide (nmol/L)</td>
<td>0.95 ± 0.11</td>
<td>0.94 ± 0.12</td>
<td>0.838</td>
<td>0.88 ± 0.10</td>
<td>0.96 ± 0.14</td>
<td>0.719</td>
</tr>
<tr>
<td>Insulin-glucose ratio</td>
<td>22 ± 5.1</td>
<td>23 ± 6.3</td>
<td>0.614</td>
<td>20 ± 4.4</td>
<td>23 ± 6.4</td>
<td>0.841</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.5 ± 0.9</td>
<td>3.3 ± 0.8</td>
<td>0.719</td>
<td>3.1 ± 0.8</td>
<td>3.3 ± 0.8</td>
<td>0.795</td>
</tr>
</tbody>
</table>

PH, protein hydrolysate; Pla, placebo; HOMA-IR, Homeostasis Model Assessment of insulin resistance; Values are mean ± SEM.

![Fig. 2.](image-url)
Fig. 3. Values of fasting, mean postprandial and mean daily plasma glucose concentrations on days 2–7 of twice daily ingestion of protein hydrolysate (PH) or placebo drink in patients with gestational diabetes.

* P<0.05
and glucose responses in patients with GDM. In previous studies, the effects of a single dose of PH were investigated in type 2 diabetic patients or healthy volunteers. A strength of the current study is that it was performed under relatively well-controlled conditions: on test days (days 1 and 8 of the study), the volunteers had to consume a standardised breakfast. We must acknowledge some limitations of our study. Firstly, the study was performed in a single centre and the sample size of this study was limited. However, the sample size of the present study is comparable to previous studies and a power calculation demonstrated that a sample size of n = 21 was sufficient to detect our primary endpoint. Secondly, due to concerns of adverse gastrointestinal effects in a pregnant population, the selected dose of the PH was relatively low and divided over two meals, consequently more studies are need to determine the tolerability of higher doses of PH in GDM patients.

5. Conclusion

In a cohort of GDM patients we have shown that 8.5 g of PH supplement twice daily is not associated with an increase in plasma insulin or C-peptide levels, although a small decrease in average daily glucose levels was found, suggesting an increase in insulin sensitivity. More research is necessary to determine whether higher doses of PH in GDM patients can be tolerated and result in better management of GDM.

Author's contributions

L. Saleh included patients, acquired, analysed and interpreted the data and wrote the manuscript. N.L. Schrier analysed the data, M.J. Bruins contributed to the design of the study and reviewed/edited manuscript. E.A.P. Steegers contributed to the conception of the study and reviewed the manuscript. A.H. van den Meiracker contributed to the design of the study and reviewed the data, L. Saleh was the principal investigator and contributed to the discussion and W. Visser was the principal investigator and contributed to the design of the study, contributed to the discussion and W. Visser was the principal investigator and contributed to the discussion and E.A.P. Steegers contributed to the conception of the study and reviewed the manuscript and contributed to the discussion.

Conflict of interest

None.

Acknowledgements

This work was performed at Department of Obstetrics and Gynaecology, Erasmus MC. The authors thank research assistants Joke van Rhee and Titia de Winter for their preparation during the different phases of the study. This research was funded by DSM Food Specialties, Delft, The Netherlands.

References


Table 3

<table>
<thead>
<tr>
<th>Side-effect</th>
<th>PH</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>26</td>
<td>26</td>
<td>–</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
<td>12%</td>
<td>0.187</td>
</tr>
<tr>
<td>Belching</td>
<td>11</td>
<td>46%</td>
<td>0.419</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>2</td>
<td>8%</td>
<td>0.504</td>
</tr>
<tr>
<td>Abdominal fullness</td>
<td>4</td>
<td>17%</td>
<td>0.814</td>
</tr>
<tr>
<td>Flatulence</td>
<td>4</td>
<td>17%</td>
<td>0.802</td>
</tr>
<tr>
<td>Constipation</td>
<td>4</td>
<td>17%</td>
<td>0.329</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
<td>8%</td>
<td>0.504</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>5</td>
<td>21%</td>
<td>0.848</td>
</tr>
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