Influence of whey protein and its hydrolysate on prehypertension and postprandial hyperglycaemia in adult men

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ABSTRACT

The effects of pre-meal consumption of whey protein isolate (WPI) and its hydrolysate (WPH) on blood pressure in pre-hypertensive and post-meal plasma responses in type 2 diabetic subjects were investigated. In a placebo-controlled double-blind randomised study, ten male patients with type 2 diabetes received dosages of WPI or WPH (0, 0.1, 0.2 and 0.4 g kg\(^{-1}\) body weight). Ingestion of 0.2 g kg\(^{-1}\) WPH or 0.4 g kg\(^{-1}\) WPI markedly promoted insulin secretion leading to a major reduction \((p < 0.01)\) in post-challenge plasma glucose response. Plasma insulin concentration increased significantly \((p < 0.05)\) only in the 0.4 g kg\(^{-1}\) WPH trial concomitant with returning the glucose level to the normal range 2 h after the meal. Neither WPH nor WPI (21 g day\(^{-1}\)) caused a significant reduction \((p > 0.05)\) in systolic blood pressure and diastolic blood pressure of pre-hypertensive subjects over the one-week administration.

1. Introduction

Growing demands for nutraceuticals have strongly encouraged the food industry to develop novel functional food ingredients. The multifunctional properties of milk-derived bioactive peptides offer considerable potential for development of nutraceutical ingredients (Korhonen, 2009). Commercial casein hydrolysates with antihypertensive activity (e.g., Calpis\(^{®}\) and Evolus\(^{®}\)) and glycaemic control properties (e.g., Insuvital\(^{®}\)) are already available, although in limited markets. Whey protein hydrolysates (WPHs) have been proven to exert an antihypertensive effect in vitro and/or in vivo (Hernández-Ledesma, Contreras, & Recio, 2011). Likewise, there is increasing evidence that WPHs could manage postprandial hyperglycaemia in type 2 diabetic patients through stimulation of insulin secretion (Jakubowicz & Froy, 2013; Manders et al., 2006).

We recently optimised the hydrolysis conditions of whey proteins digested by a protease preparation from Aspergillus oryzae to achieve high angiotensin converting enzyme (ACE)-inhibitory and antioxidant properties in vitro (Goudarzi, Madadlou, Mousavi, & Emam-Djomeh, 2012). It is of interest to evaluate the efficiency of this WPH in regulating blood pressure and post-meal plasma glucose level in vivo. The present study was therefore conducted to examine the effect of consumption of this WPH on blood pressure and post-meal plasma glucose and insulin concentration in pre-hypertensive and type 2 diabetic patients, respectively. The antihypertensive and anti-diabetic activities of the intact whey protein isolate (WPI) were also studied to evaluate the efficacy of hydrolysate over the whole protein.

2. Materials and methods

2.1. Materials

WPI and flavourzyme (protease from A. oryzae) were generously provided by Arla Foods (Aarhus, Denmark) and Novozymes (Kanto/Chiba, Japan), respectively.

2.2. Whey protein hydrolysis and beverage preparation

Sixteen grams of WPI was dissolved in 100 mL distilled water and kept at 4 \(^{\circ}\)C overnight to allow complete hydration. Enzymatic hydrolysis was then carried out by incubating 1.5 mg flavourzyme mL\(^{-1}\) WPI solution under optimum conditions (65 \(^{\circ}\)C, 3.9 h, pH 6.0) to achieve 74% ACE-inhibitory and antioxidant activity of 666.3 \(\mu\)M trolox equivalent g\(^{-1}\) in vitro (Goudarzi et al., 2012). Hydrolysis was terminated by heating the hydrolysate at 80 \(^{\circ}\)C for 20 min, after which time the solution was centrifuged at 20,000 \(\times\) g for 5 min. The supernatant was freeze-dried into powder and stored at –80 \(^{\circ}\)C. The whey protein beverages were prepared by dissolving 6 g WPI or WPH powder in 100 mL distilled water. The solutions

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were then pasteurized at 78 °C for 20 s and kept refrigerated until in vivo investigation.

2.3. Subjects

Ten male patients with type 2 diabetes (age, 32.4 ± 4.1 y; body mass index, 26.2 ± 1.2 kg m⁻²; fasting plasma glucose, 7.6 ± 0.5 mmol L⁻¹) and ten male patients with prehypertension (age, 26.2 ± 3.4 y; body mass index, 24.8 ± 1.6 kg m⁻²; systolic blood pressure, 130.5 ± 5.2 mm Hg; diastolic blood pressure, 84.6 ± 3.9 mm Hg) participated in the study. The pre-hypertensive subjects were not using any blood pressure-lowering medications. The diabetic patients were taking metformin (n = 2) and metformin with sulphonylurea derivations (n = 8). All subjects were informed about the nature of the beverages tested and signed informed consent was obtained from each subject. The experiments were carried out at Chamran Hospital (Boroujerd, Iran). The study protocol was approved by the hospital ethics committee.

2.4. Study design

In a double-blind, randomised study, type 2 diabetic patients received seven different treatments including WPH beverages (0.1, 0.2 and 0.4 g kg⁻¹ body weight), WPI beverages (0.1, 0.2 and 0.4 g kg⁻¹ body weight) and a control (distilled water). Each trial was performed on a distinct day with three-day intervals between trials. The subjects were asked to keep their usual dietary and physical activity patterns during the study days. The use of glucose-lowering medications was withheld 2 days before each trial.

After a 10 h overnight fast, the patients were admitted to the hospital at 8.00 a.m. and were served the whey protein beverages. Blood samples (4 mL) were taken from each participant immediately after whey protein consumption (t = 0 min) to measure plasma glucose and insulin concentration. Thirty minutes after the ingestion of beverages, the subjects were fed a meal of 12 kcal kg⁻¹ energy (59% energy carbohydrates, 24% energy protein, 17% energy fat) and allowed to eat within another 30 min (Akhavan, Luhovyy, Brown, Cho, & Anderson, 2010). The blood samples were collected at 30 min intervals from 0 to 180 min and analysed for glucose and insulin concentration to evaluate the insulinitropic effect of whey protein or its hydrolysate on post-meal hyperglycaemia in diabetic patients. The blood samples were centrifuged at 1000 × g at 4 °C for 15 min (Morifuji et al., 2012) to obtain the plasma. Plasma glucose and insulin concentration were measured by colourimetric glucose oxidase (Yuen & McNeill, 2000) and enzyme immunoassay (Andersen, Dinesen, Jørgensen, Poulsen, & Røder, 1993) methods, respectively.

The pre-hypertensive subjects were treated with 24 g whey protein or its hydrolysate per day in a one-week study period. Two experiments were separated by an interval of 7 days. The subjects were counselled to consume the same food on 3 days before the study and throughout the study days. At each daily visit, the participants were presented with beverages for the next day including two 200 mL bottles of WPI or WPH solutions to be consumed 1 h before main meals (breakfast and lunch). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured on days 0, 1, 2, 4 and 7. The measurements were taken 3 h after the lunch between 3.00 and 5.00 p.m.

2.5. Statistical analyses

The results were expressed as means ± standard deviation and were analysed by one-way analysis of variance (ANOVA) using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA). Dunnett’s multiple comparison test was carried out to examine the effect of WPI or WPH consumption on the trend of blood pressure and plasma glucose and insulin changes from the baseline (t = 0 min) within α = 0.05 or 0.01 significance levels. Significant differences between blood pressure at the time intervals as well as among mean postprandial plasma responses on different treatment were determined using Duncan’s multiple range test. A retrospective analysis was used after collecting data to understand the power of tests performed. The statistical power of tests (p), the probability of not missing an effect, due to sampling error, when there really is an effect to be found (Hoenig & Heisey, 2001), were calculated using Minitab version 15.1.10 (Minitab Inc., USA).

3. Results and discussion

3.1. Anti-diabetic study

The postprandial plasma glucose and insulin levels of type 2 diabetic patients ingested with different doses of WPH or WPI are presented in Tables 1 and 2. The plasma glucose concentration of control subjects peaked within 2 h after the meal (t = 120 min) and remained at a statistically significant higher level than baseline (t = 0) during the study period. There was also a similar trend for plasma insulin level changes. This implies that the insulin has not effectively been able to take the glucose from blood to cells. This has most probably stemmed from a physiological condition usually occurring in overweight individuals (body mass index > 25 kg m⁻²) (Flint et al., 2007) in which the cells resist the normal action of insulin to deliver glucose to cells (Hulver & Dohm, 2004). As a result, a high concentration of glucose exists in the
bloodstream in the presence of an elevated insulin response (Matsuda & Defronzo, 1999). 

Treating with 0.1 g kg\(^{-1}\) WPH, 0.1 g kg\(^{-1}\) WPI or 0.2 g kg\(^{-1}\) WPI caused a significant reduction in plasma insulin at t = 150 min (Table 2), which is attributed to improvement of insulin resistance of cells induced by whey proteins. Pal, Ellis, and Dhalwiya (2010) observed a remarkable decrease in fasting insulin level and homeostasis model assessment of insulin resistance scores of overweight/obese individuals treated with whey protein isolate. The results obtained in the present study, however, reveal that the decreased insulin concentration was not accompanied by a statistically significant reduction in plasma glucose level (Table 1) suggesting that the insulin level was not sufficient to transport a large amount of glucose into the cells.

Overall, the subjects supplemented with 0.1 g kg\(^{-1}\) WPH, 0.1 g kg\(^{-1}\) WPI or 0.2 g kg\(^{-1}\) WPI showed no significant changes in 2 h post-meal blood insulin and glucose concentration compared with the control group (Fig. 1). Consistent with our results, Jonker et al. (2011) reported that ingestion of a low dose of casein hydrolysate (6 g) did not affect the blood responses of type 2 diabetics. As seen in Fig. 1, the patients consumed the 0.2 g kg\(^{-1}\) WPH or 0.4 g kg\(^{-1}\) WPI experienced a notable, but not statistically significant, promotion in 2-h postprandial blood insulin level, which in turn led to a major reduction (p < 0.01) in their post-challenge blood glucose values in comparison with the control group. It is argued that specific amino acids as well as bioactive peptides either present in WPH or produced during gastrointestinal digestion of WPI or WPH enhanced insulin secretion through an effect on the Ca\(^{2+}\) channels and Ca\(^{2+}\)-influx of islet \(\beta\)-cells, a pancreatic cell responsible for storage and release of insulin (Bosscher et al., 2009). The results demonstrated that 0.4 g kg\(^{-1}\) WPH was the only treatment that induced a statistically high insulin secretion (p < 0.01 versus control) (Fig. 1) and hereby returned the blood glucose level to the normal range (<7.8 mmol L\(^{-1}\); American Diabetes Association, 2006) 2 h after the meal (Table 1). Moreover, the subjects that received 0.4 g kg\(^{-1}\) WPH had no significant rise (p > 0.05) in blood glucose level during the postprandial period (Table 1) possibly due to continuous delivery of glucose by highly elevated insulin into the cells. This is in line with the results of van Loon, Kruijshoop, Verhagen, Saris, and Wagenmakers (2000), who found that doubling the dose of wheat protein hydrolysate (0.2–0.4 g kg\(^{-1}\) h) effectively increased the post-exercise insulin response in men. The differences between treatments on plasma responses corresponded well with the results of Manders et al. (2006). However, the differences detected should also be clinically relevant. The plasma glucose and insulin concentration changes of individuals treated with 0.4 g kg\(^{-1}\) WPH during 2 h postprandial period is almost similar to results of an oral glucose tolerance test (OGT) in subjects with normal glucose tolerance (Matsuda & Defronzo, 1999). Furthermore, the diabetic patients who consumed 0.2 g kg\(^{-1}\) WPH or 0.4 g kg\(^{-1}\) WPI showed postprandial plasma glucose and insulin changes similar to pre-diabetic subjects (Matsuda & Defronzo, 1999). These observations can be

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>t = 0 min</th>
<th>t = 30 min</th>
<th>t = 60 min</th>
<th>t = 90 min</th>
<th>t = 120 min</th>
<th>t = 150 min</th>
<th>t = 180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>78.2 ± 19.8</td>
<td>75.4 ± 22.8</td>
<td>110.9 ± 36.6</td>
<td>174.2 ± 38.3</td>
<td>281.1 ± 68.6**</td>
<td>234.5 ± 72.5*</td>
<td>202.9 ± 84.2*</td>
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<tr>
<td>WPI</td>
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</tr>
<tr>
<td>0.1 g kg(^{-1})</td>
<td>85.7 ± 20.0</td>
<td>78.3 ± 34.1</td>
<td>115.7 ± 42.6</td>
<td>171.4 ± 56.7</td>
<td>267.5 ± 45.4**</td>
<td>234.1 ± 67.9*</td>
<td>177.6 ± 84.3</td>
</tr>
<tr>
<td>0.2 g kg(^{-1})</td>
<td>92.0 ± 31.4</td>
<td>100.2 ± 59.4</td>
<td>146.8 ± 93.4</td>
<td>246.9 ± 68.2*</td>
<td>338.4 ± 89.2**</td>
<td>276.7 ± 94.9**</td>
<td>216.6 ± 57.9</td>
</tr>
<tr>
<td>0.4 g kg(^{-1})</td>
<td>76.8 ± 25.1</td>
<td>92.0 ± 34.5</td>
<td>169.9 ± 64.3</td>
<td>286.0 ± 86.4*</td>
<td>368.6 ± 74.3**</td>
<td>468.9 ± 81.1**</td>
<td>301.5 ± 84.9**</td>
</tr>
<tr>
<td>WPH</td>
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</tr>
<tr>
<td>0.1 g kg(^{-1})</td>
<td>65.6 ± 28.4</td>
<td>85.6 ± 28.9</td>
<td>145.6 ± 44.3</td>
<td>276.5 ± 75.6*</td>
<td>324.5 ± 62.7***</td>
<td>254.3 ± 92.1*</td>
<td>180.6 ± 66.7</td>
</tr>
<tr>
<td>0.2 g kg(^{-1})</td>
<td>85.2 ± 22.4</td>
<td>120.5 ± 45.2</td>
<td>210.9 ± 45.6</td>
<td>388.2 ± 84.1**</td>
<td>471.5 ± 102.3**</td>
<td>440.0 ± 88.5**</td>
<td>311.2 ± 90.1**</td>
</tr>
<tr>
<td>0.4 g kg(^{-1})</td>
<td>75.0 ± 18.7</td>
<td>143.5 ± 62.4</td>
<td>380.2 ± 112.2</td>
<td>721.7 ± 125.2***</td>
<td>686.3 ± 170.8***</td>
<td>549.8 ± 134.8**</td>
<td>280.2 ± 124.5</td>
</tr>
</tbody>
</table>

* Data are means ± SD (n = 10); a single asterisk indicates different from the baseline (t = 0 min) at p < 0.05 significance level, a double asterisk indicates different from the baseline (t = 0 min) at p < 0.01 significance level.
regarded as clinically relevant improvements. It is worth noting that WPH showed noticeably better effectiveness in blood glucose control than intact whey protein. The higher contribution of WPH in hyperglycaemia management might be from faster digestion and quicker availability of its insulinotropic peptides and amino acids in the blood (Jonker et al., 2011).

3.2. Antihypertensive study

The changes in SBP and DBP of pre-hypertensive subjects treated with whey protein or its hydrolysate are reported in Fig. 2. Mizuno et al. (2005) reported that 1.8 mg daily administration of casein hydrolysate obtained by an A. oryzae protease resulted in a reduction of 1.8 and 1.4 mm Hg in SBP and DBP of pre-hypertensive subjects over a one-week period, respectively. Results showed that SBP and DBP declined from the baseline (t = 0 day) by 1.8 ± 1.0 and 1.4 ± 1.2 mm Hg, respectively, after one week of consumption of intact WPI (Fig. 2), whilst, WPH intake for the identical time period resulted in a reduction of 5.4 ± 2.1 mm Hg in SBP and 2.5 ± 1.5 mm Hg in DBP. It has been estimated that each 2 mm Hg reduction of SBP is associated with a 6% decrease in stroke mortality (Cornelissen & Fagard, 2005). Likewise, a 5 mm Hg decrease in DBP would reduce the risk for cardiovascular diseases by 16% (Ricci, Artacho, & Olalla, 2010).

Statistical analysis revealed that the recorded changes in SBP and DBP of subjects during one-week consumption of WPI or WPH were not significant (Fig. 2; p > 0.05). This suggests that intake of whey proteins in either intact or hydrolysed form do not modulate blood pressure in pre-hypertensive male subjects. However, Pins and Keenan (2006) in a 6 week controlled study indicated that consumption of 20 g day superscript −1 WPH caused a significant decrease of 8 ± 3.2 mm Hg in SBP and 5.5 ± 2.1 mm Hg in DBP of subjects with mild hypertension by the end of first week of treatment which maintained unchanged over the rest of study duration.

The results of statistical power calculation of tests are presented in Table 3. The higher the power, the stronger the probability of correctly accepting or rejecting the null hypothesis (null hypothesis refers to a position in which there is no statistically relationship between two measured phenomena; Hoenig & Heisey, 2001). In a prospective study which is used before collecting data to estimate sufficient sample size, the power (p) is normally set at 0.8 (80%) or higher (Röhrig, du Prel, Wachtlin, Kwiecien, & Blettner, 2010). It implies that the sample sizes required to detect differences in means in the present study were adequate (Table 3).

4. Conclusion

The whey protein hydrolysed with a protease preparation from A. oryzae showed a considerably higher effect on glycaemic control compared with intact WPI, as diabetic patients treated with 0.4 g kg superscript −1 WPH found their 2-h postprandial blood glucose level fell within the range of healthy people. The intact whey proteins and their hydrolysates do not significantly modulate the blood pressure in pre-hypertensive male subjects.

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References


