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The thermogenic and metabolic effects of protein hydrolysate with or without a carbohydrate load in healthy male subjects

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Abstract

High-protein diets are beneficial in weight maintenance because of their satiating and thermogenic effects. These effects may be partly mediated by the hormonal effects of proteins. This study investigated the effect of soy protein hydrolysate (SPH) with and without a carbohydrate pre- and afterload on energy metabolism and hormonal secretion in 8 healthy nonobese subjects. In an additional trial, pea protein hydrolysate was compared to SPH, both with a carbohydrate afterload. The study had a single-blind crossover design. In all cases, 0.4 g protein and/or carbohydrate per kilogram of body weight was tested. Diet-induced thermogenesis (DIT) was measured by ventilated hood measurements, and postprandial blood samples were drawn over 3 hours. Soy protein hydrolysate consumption induced a higher DIT than a carbohydrate (CHO) load. Both conditions induced similar insulin responses. Soy protein hydrolysate induced a glucagon, but no glucose, response; whereas CHO induced a glucose, but no glucagon, response. Soy protein hydrolysate with a CHO pre- or afterload induced similar DIT and insulin responses. No glucose response was found when SPH preceded the CHO load. Total glucagon responses were similar with CHO as pre- and afterload, but time courses were different. Pea protein hydrolysate with a CHO afterload induced both higher insulin and glucagon responses (area under the curve) than SPH with CHO afterload, but DIT was similar in both conditions. In conclusion, this study shows that the larger DIT after protein than after CHO may be related to the glucagon response that is induced by protein but not by CHO; that the protein-induced DIT and glucagon response are not influenced by a CHO pre- or afterload; and that protein ingestion can fully prevent the plasma glucose increase associated with CHO when CHOs are ingested after proteins.

1. Introduction

In obesity research, high-protein diets are of special interest because they induce weight loss [1,2] and reduce weight regain after a period of weight loss [3]. This beneficial effect may be due to the satiety-inducing, thermogenic, and/or hormonal effects of dietary proteins. Lejeune et al [4] showed that a 4-day high-protein diet (30 En% protein) in healthy female subjects increased 24-hour satiety, energy expenditure (EE) (mainly sleeping metabolic rate and diet-induced thermogenesis [DIT]), and fat oxidation compared with a low-protein diet (10 En% protein). Furthermore, a high-protein meal tends to induce higher DIT compared to a fat or carbohydrate meal with similar energy density [5,6]. The underlying mechanisms remain unclear. Proteins stimulate insulin secretion by pancreatic beta cells [7,8] and induce glucagon secretion by pancreatic alpha cells [8,9]. The most studied function of glucagon is its counter-regulatory response to hypoglycemia. In this regard, glucagon stimulates glucose output by increasing liver glycogenolysis and gluconeogenesis [10]. Next to its function in glucose metabolism, glucagon is also thought to play a role in lipolysis and amino acid metabolism that both have different effects on EE. Nair [11] showed in 1987 that glucagon infusion can increase EE by increasing gluconeogenesis and protein oxidation. A stimulatory effect of glucagon on lipolysis has been suggested [12,13], but inconsistent findings have been reported [12-15]. Previous studies did not distinguish the effects of dietary carbohydrate and protein on glucagon and insulin secretion or their mutual influences [8,9,16].

The purpose of the present study was to compare the thermogenic effects and hormonal responses to equi-energetic protein hydrolysate and carbohydrate loads. We hypothesized that DIT would be higher after the protein

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Table 1
Amino acid composition of soy and PPH

Amino acid	Soy	Pea
Asparagine/aspartic acid	12.1	11.7
Serine	5.2	5.7
Glutamine/glutamic acid	19.7	19.9
Histidine	2.5	2.4
Glycine	4.3	4.0
Threonine	3.9	4.0
Arginine	7.4	9.8
Alanine	4.4	5.4
Tyrosine	3.1	3.7
Valine	3.9	3.9
Methionine	1.2	0.9
Isoleucine	3.7	3.4
Phenylalanine	4.9	4.6
Tryptophan	1.3	0.4
Leucine	7.5	7.3
Lysine	6.6	8.4
Proline	6.4	4.0
Cysteine	2.2	0.6

Values are expressed as percentage of total dry product.

hydrolysate than after the carbohydrate load and that this would be associated with a higher glucagon response. In addition, we investigated the interaction of the protein hydrolysate load with a carbohydrate pre- or afterload with respect to DIT and hormonal responses. The protein source used in these experiments was soy. However, in one of the trials, pea protein hydrolysate (PPH) was used to study possible differences between protein sources. Protein hydrolysates were used because there are indications that they are metabolized faster than intact protein [17].

2. Subjects and methods

2.1. Subjects

Eight healthy nonobese male subjects participated in this study (means ± SEM [range]: age, 28.5 ± 3.6 years [19-48 years]; weight, 75.9 ± 2.2 kg [66-82 kg]; height, 1.81 ± 0.02 m [1.75-1.95 m]; body mass index, 23.3 ± 0.7 kg/m^2 [21.5-26.8 kg/m^2]). Subjects underwent a brief medical screening, including a medical history, and a fasting blood sample was collected. Subjects were excluded from the study if they were not weight stable over the past 2 months before enrollment, when fasting blood glucose levels were greater than 5.5 mmol/L, when body mass index is greater than 27 kg/m^2, or when they were using medication. All subjects were informed about the nature and risks of the experimental procedures and their informed consent was obtained. The local medical ethical committee approved this study.

2.2. Experimental design

The study consisted of 5 trials in which drinks containing soy protein hydrolysate (SPH), carbohydrate, and SPH with carbohydrate as pre- or afterload were tested. Furthermore, PPH with a carbohydrate afterload was tested. All beverages were artificially sweetened and lemon flavored to make differences in taste as small as possible. Beverages were offered randomly (by means of Latin-square randomization) and in untransparent mugs to obtain a single-blind crossover study. Two testing days were separated by at least 2 consecutive washout days. The period to complete all trials varied for subjects between 14 and 33 days with a mean of 23 days. On testing days, subjects came by car or by public transport to the laboratory after an overnight fast. They were asked to avoid heavy physical activity and to keep their eating pattern as constant as possible the day before the trials.

2.3. Protocol

After at least 10 hours fasting, subjects reported at approximately 8:00 AM at the laboratory where the basal metabolic rate was measured by indirect calorimetry using an open-circuit, ventilated-hood system with subjects lying in supine position. Before BMR measurements were started, subjects rested for at least 20 minutes in supine position.

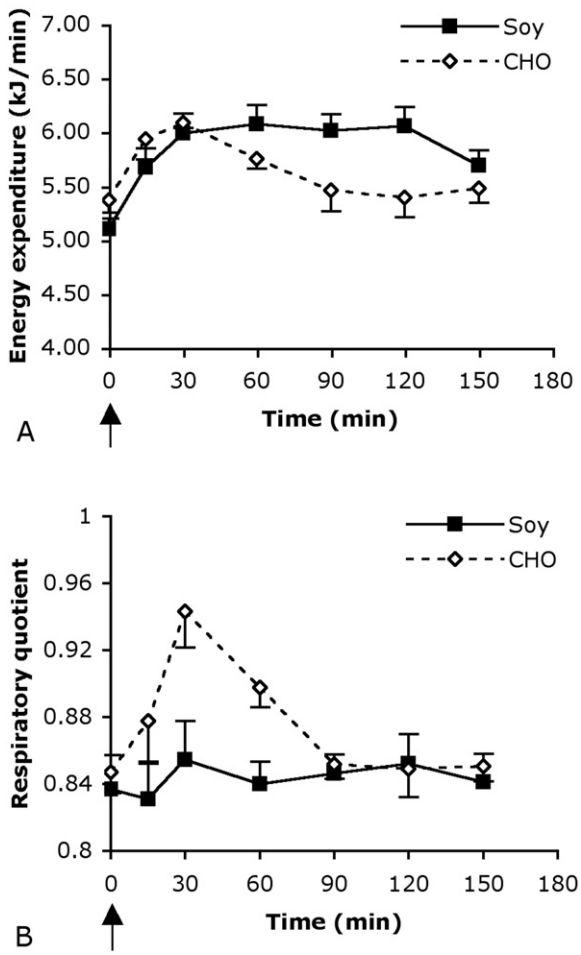

Fig. 1. Change in EE (A) and RQ (B) over time after SPH or carbohydrate consumption. ↑ indicates moment of consumption (at t = 0 minute).

Table 2
Total AUC for parameters after ingestion of SPH and CHO

	Soy	CHO	*P*
Glucose	−16.3 ± 8.8	48.8 ± 15.9	.02
Insulin	1257.0 ± 188.6	1412.8 ± 332.5	.89
Glucagon	5575.6 ± 657.6	−185.6 ± 305.2	.00
FFAs	−14116.0 ± 6119.7	−11356.0 ± 3512.6	.48
TGs	−15476.2 ± 5446.7	−10177.0 ± 6202.0	.48
Lactate	−3.6 ± 7.1	67.9 ± 8.7	.01
Urea	65.8 ± 23.9	−50.8 ± 6.0	.01
EE	124.2 ± 11.2	38.8 ± 7.6	.01
RQ	0.99 ± 2.05	3.48 ± 1.77	.33

Values are expressed as mean ± SEM.

Basal metabolic rate was calculated from oxygen consumption and carbon dioxide production over 30 minutes using the formula of Weir [18]. Respiratory quotient (RQ) was calculated as the ratio of the volume of carbon dioxide expired to the volume of oxygen consumed. After BMR measurements a Teflon catheter (Baxter BV, Utrecht, The Netherlands) was inserted into an antecubital vein and a resting blood sample was drawn ($t = 0$). Then subjects were offered a test drink that they had to consume as fast as possible and at least within 5 minutes. After finishing the test drink, DIT was determined by means of continuous indirect calorimetry over 2.5 hours. During this period, subjects were not allowed to eat or drink. Diet-induced thermogenesis measurements were interrupted once (after blood sampling at $t = 90$) on all experimental days to allow subjects to have a sanitary stop. Blood samples were drawn 15, 30, 60, 90, 120, and 150 minutes after finishing the test drink. In case of the trials with a carbohydrate pre- or afterload, DIT

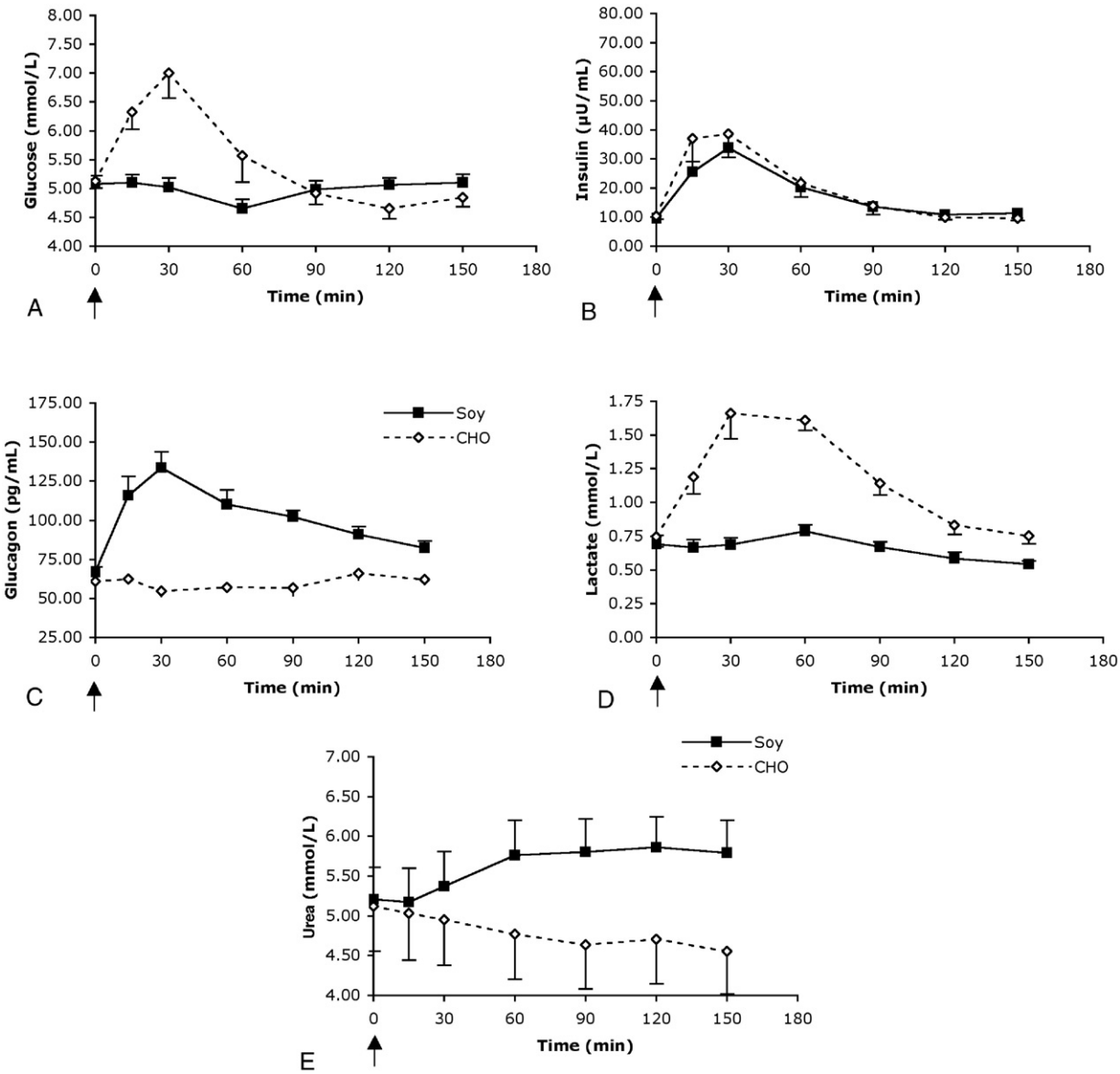

Fig. 2. Change in glucose (A), insulin (B), glucagon (C), lactate (D), and urea (E) concentration over time after SPH or carbohydrate consumption. ↑ indicates moment of consumption (at $t = 0$ minute).

measurements were interrupted shortly after 30 minutes for consumption of the second test drink.

2.4. Beverages

All beverages (approximately 250 mL) offered to the subjects consisted of 0.4 g protein hydrolysate per kilogram of body weight or of 0.4 g maltodextrin per kilogram of body weight (AVB, Veendam, The Netherlands). This amount of proteins is comparable to the protein load ingested when eating a standard Dutch lunch [19]. The pH of all beverages was adjusted to 3.4 in a standard way with citric acid to optimize the taste profile of the flavoring compound (Lemon Flavor, Quest, Naarden, The Netherlands) (Table 1).

2.5. Blood analyses

Blood was collected in EDTA-containing tubes for glucose, insulin, glucagon, free fatty acids (FFAs), triglycerides (TGs), lactate, and urea analysis. EDTA blood to which aprotinin (5 kIU/mL blood; Sigma-Aldrich Chemie BV, Zwijdrecht, The Netherlands) was added was used for glucagon analysis. After collection the blood sample was centrifuged at 1000*g* at 4°C for 10 minutes. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at −45°C. Glucose, FFAs, lactate, urea, and TGs were analyzed with the COBAS FARA semiautomatic analyzer (Roche Diagnostica, Basel, Switzerland). Insulin was quantitated by radioimmunoassay (Human Insulin-Specific RIA Kit, LINCO Research, St Charles, MO) as was glucagon (Glucagon RIA Kit, LINCO Research).

2.6. Statistics

All data are expressed as means ± SEM (n = 8). Statistical analysis was performed using SPSS for Mac OS X software (SPSS, Chicago, IL). Statistical analyses were done separately for the trials in which a single load was tested, for the trials in which SPH was combined with a carbohydrate pre- or afterload, and for the trials in which soy and PPH were followed by a carbohydrate afterload. Plasma glucose, insulin, glucagon FFAs, lactate, urea, and TG responses were calculated as the total area under the curve (AUC) (AUC above − AUC below baseline [t = 0]). Differences in AUC over 150 minutes were assessed by Wilcoxon matched-pairs signed-rank tests. For each trial the time point that peak concentrations for a certain parameter were measured for most subjects was chosen as time to peak. Δ values (concentration at time point x − baseline value) were calculated and differences in Δ peak values were also tested by means of Wilcoxon matched-pairs signed-rank tests. Multiple linear regression analysis was used to determine the contribution of insulin and glucagon to EE. A model was used in which EE was the dependent variable and insulin and/or glucagon responses was the independent variable. Furthermore, the model was corrected for subject, trial, and time point. At a P level of .05 or smaller, results were considered significantly different.

3. Results

3.1. Single SPH load vs single carbohydrate load

Fig. 1A shows that both conditions induced similar peak values for EE at t = 30 minutes (P = .32), but although EE decreased after t = 30 minutes in the CHO condition, it remained increased until t = 120 minutes in the SPH condition. Δ values for EE (Δ EE) were significantly higher after SPH than after CHO from t = 60 minutes onward ($P \leq$ .025). This difference resulted in a significantly higher AUC for EE after SPH consumption (P = .012). No significant differences were found in AUC values for RQ (P = .33) (Table 2), but the Δ values for RQ (at t = 30 minutes) (Δ RQ) was significantly higher in the CHO condition (P = .035).

Fig. 2A shows that a single CHO load induced a plasma glucose response with peak concentrations at t = 30 minutes, whereas a single equi-energetic load of SPH did not affect plasma glucose. The AUC for glucose was significantly higher in the CHO compared to the protein condition (P = .017) (Table 2).

Both conditions induced similar insulin responses (expressed as AUC, P = .89) (Table 2). The CHO load did not induce a glucagon response in contrast to the SPH

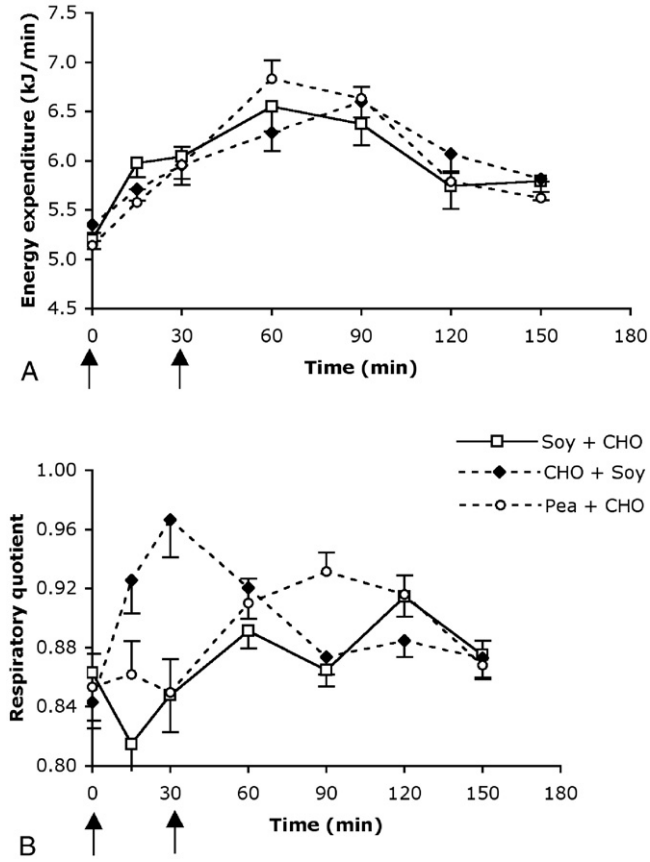

Fig. 3. Change in EE (A) and RQ (B) over time after SPH consumption with a carbohydrate pre- or afterload and after PPH consumption with a carbohydrate afterload. ↑ indicates moment of consumption (at t = 0 and at t = 30 minutes).

load with peak concentrations at t = 30 minutes. The AUC values for glucagon differed significantly between CHO and SPH ($P < .01$), as did Δ values (Δ glucagon) at t = 30 minutes (P = .012).

The AUC values for FFAs and TGs did not differ significantly (P = .48 and P = .48, respectively). The AUC for lactate was significantly higher after CHO consumption (P = .012), whereas the AUC for urea was significantly higher after SPH consumption (P = .012) (Table 2).

3.2. Soy protein hydrolysate with a carbohydrate pre- or afterload

Both conditions induced increases in EE and RQ, which, expressed as AUC, did not differ significantly (P = .12 and P = .33, respectively) (Table 2). Peak values for EE were not significantly different (P = .07 at t = 60 minutes; P = .779 at t = 90 minutes), and no significant differences were found for Δ EE at t = 60 minutes or t = 90 minutes between conditions (P = .069 and P = .779, respectively). Peak values in RQ were found at t = 30 minutes with the CHO preload, whereas in case of the CHO afterload, RQ values reached peak values at t = 120 minutes (Fig. 3B). At t = 30 minutes, Δ RQ was significantly higher with the CHO preload (P = .028).

Fig. 4A shows that SPH preceded by a CHO load induced a plasma glucose response with peak concentrations at t = 30 minutes, whereas a SPH load followed by a CHO load did not affect plasma glucose concentrations. Δ glucose at t = 30 minutes was significantly higher with the CHO preload (P = .012). The AUC values for both conditions differed significantly (P = .012) (Table 3).

The AUC values of insulin responses did not differ significantly (P = .263), and peak insulin concentrations were found at t = 60 minutes in both conditions and also

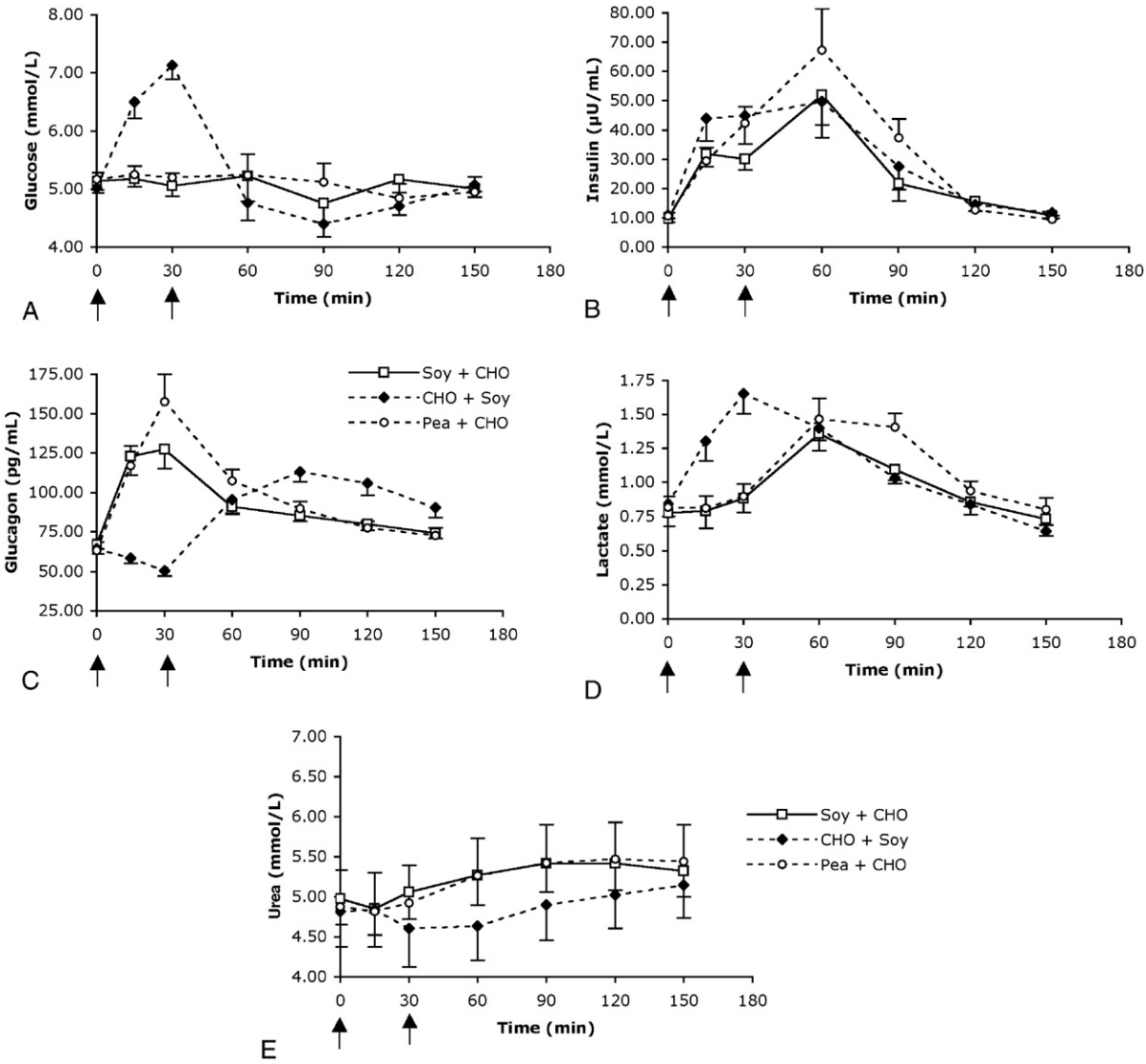

Fig. 4. Change in glucose (A), insulin (B), glucagon (C), lactate (D), and urea (E) concentration over time after SPH consumption with a carbohydrate pre- or afterload and after PPH consumption with a carbohydrate afterload. ↑ indicates moment of consumption (at t = 0 and at t = 30 minutes).

Table 3
Total AUC for parameters after ingestion of SPH with CHO as pre- or afterload

	Soy + CHO	CHO + soy	*P*
Glucose	−10.9 ± 22.4	34.6 ± 12.6	.01
Insulin	2654.0 ± 577.7	3062.4 ± 691.7	.26
Glucagon	3955.7 ± 605.2	3625.7 ± 490.6	.67
FFAs	−16599 ± 2794	−13324 ± 2915	.48
TGs	−4515.7 ± 3928.1	−7879.0 ± 6336.3	.78
Lactate	31.5 ± 6.3	44.3 ± 9.4	.78
Urea	40.7 ± 11.3	−5.6 ± 8.7	.02
EE	133.1 ± 13.6	112.6 ± 16.7	.12
RQ	1.75 ± 4.31	7.58 ± 1.81	.33

Values are expressed as mean ± SEM.

were not significantly different (P = .36). Glucagon concentrations did not change significantly after CHO preload consumption and increased when SPH was consumed 30 minutes later, reaching peak values at t = 90 minutes. When SPH was consumed first, a glucagon response was induced immediately, reaching peak values at t = 30 minutes. The AUC values for glucagon were not significantly different between the 2 conditions (P = .67) (Table 3), but the time course of the glucagon responses was clearly different (Fig. 4C).

The AUC values for FFAs, TGs, and lactate did not differ significantly between conditions (P = .48, P = .78, and P = .78, respectively). The AUC for urea was significantly lower when SPH consumption was preceded by the CHO load compared with the situation in which CHO was given as an afterload (P = .017) (Table 3).

3.3. Soy vs PPH with a CHO afterload

Energy expenditure and RQ responses expressed as AUC values did not differ between soy and pea (P = .21 and P = .67, respectively) (Table 4).

Similar to SPH, PPH prevented the CHO-induced increase in plasma glucose concentration (P = .99) (Table 4).

Pea protein hydrolysate induced significantly higher insulin and glucagon responses than SPH expressed as AUC values (P = .017 and P = .012, respectively). The Δ glucagon value (at t = 30 minutes) was significantly higher after PPH than after SPH (P = .05).

The AUC values for FFAs, TGs, lactate, and urea did not differ significantly between conditions (Table 4).

3.4. Association between insulin and/or glucagon and EE

Regression analyses were done to study the association between insulin and/or glucagon and EE. Multiple linear regression analyses over all trials with EE as dependent variable showed that the model corrected for subject, trial, and time point but, without insulin or glucagon concentrations, explained 0.7% of the variability in EE. When glucagon was included in this model, it explained 13.4% of the variability (partial correlation of 0.361, P < .001), and inclusion of insulin in the model explained 27.9% of the variability (partial correlation of 0.526, P < .000). When both glucagon and insulin were included in the model, it explained 33.2% of the variability in EE.

4. Discussion

In the present study, we showed that SPH induced a higher thermogenic response than an equi-energetic CHO drink and was associated with a higher glucagon response and a similar insulin response. Furthermore, we showed that the protein-induced DIT is not influenced by a CHO pre- or afterload. Interestingly, this study demonstrated that when a CHO load was preceded by protein hydrolysate consumption, the rise in plasma glucose expected after CHO consumption did not occur. Finally, our results demonstrate that PPH induced a higher glucagon response than SPH.

4.1. Single SPH load vs single carbohydrate load

Soy protein hydrolysate caused a significantly higher DIT than the equi-energetic maltodextrin load (Fig. 1). A higher EE after a single protein load or a mixed high-protein meal compared to an equi-energetic single carbohydrate, fat, or mixed low-protein meal has been demonstrated before [6,20-23]. In accordance with Crovetti et al [24], the difference in DIT between a protein load and a CHO load was not due to the differences in the initial rise in EE but to a prolonged increase in EE after SPH ingestion. High-protein meals are associated with increased postabsorptive protein synthesis [25], which has a high metabolic cost [26]. In addition, a thermogenic effect of glucagon has been demonstrated previously by means of glucagon infusion experiments [11]. It was proposed that the increased EE during glucagon infusion was mainly related to stimulation of gluconeogenesis and ureagenesis by glucagon [11]. After SPH consumption we indeed found significantly higher glucagon and urea responses (as AUC) compared to CHO consumption.

We found that the insulin response was similar after SPH and CHO ingestion (Fig. 2). This is in agreement with Nair et al [21] who showed that single equi-energetic protein and carbohydrate loads induced similar insulin responses. In contrast, the group of Nuttall and Gannon [16,27,28] who compared the effect of ingestion of 50 g glucose in a drink or

Table 4
Total AUC for parameters after ingestion of soy and PPH with a CHO afterload

	Soy + CHO	Pea + CHO	*P*
Glucose	−10.9 ± 22.4	−10.7 ± 20.1	.99
Insulin	2654.0 ± 577.7	3534.6 ± 588.5	.02
Glucagon	3955.7 ± 605.2	5589.2 ± 400.7	.01
FFAs	−16599 ± 2794	−14501 ± 4807	.73
TGs	−4515.7 ± 3928.1	3199.4 ± 4807.4	.26
Lactate	31.5 ± 6.3	42.2 ± 6.2	.29
Urea	40.7 ± 11.3	54.7 ± 7.6	.11
EE	133.1 ± 13.6	149.9 ± 15.8	.21
RQ	1.75 ± 4.31	6.81 ± 2.19	.67

Values are expressed as mean ± SEM.

50 g protein in the form of cooked lean hamburger (6.5% fat) on insulin concentration found that the insulin-stimulating effect of the hamburger meal was much less potent than that of the glucose drink. The difference with our findings may be because of a lower gastric-emptying rate of lean beef compared to that of a protein hydrolysate drink. In agreement with these studies [16,28], we found no significant effect of protein ingestion alone on plasma glucose levels in healthy lean subjects. Stimulation of gluconeogenesis from amino acids by glucagon is likely to have contributed to the normal glucose concentrations despite the increase in insulin after SPH consumption. In this way the body is protected from hypoglycemia after protein ingestion. Because we did not find differences in plasma FFA and TG responses and no increase in fatty acid oxidation, there is no evidence that the prolonged DIT after SPH consumption is linked to increased glucagon-induced adipose tissue lipolysis.

4.2. Soy protein hydrolysate with a carbohydrate pre- or afterload

Thermogenesis induced by SPH combined with a CHO load was not influenced by the order of consumption. In both cases, consumption of a second bolus induced an extra increase in EE and insulin (Figs. 3 and 4), and total responses were not different in both conditions (Table 3). Although AUC values for glucagon were similar in both situations (Table 3), the time course of the response was clearly different (Fig. 4). Fig. 3 shows that the rise in RQ after a CHO preload decreases after the SPH afterload similarly as after a singly CHO load, whereas RQ values remain rather low in the CHO afterload condition. Although no significant difference is found in lactate response (reflecting glycolysis), plasma urea response is significantly different between both conditions (reflecting protein turnover) (Table 3). Although urea concentrations decreased during the first hour in the CHO preload condition, they increased afterward (Fig. 4), indicating a switch in substrate oxidation.

Fig. 4 shows that a CHO pre- or afterload has a similar influence on the protein-induced insulin response. In both cases, peak values for insulin are reached at $t = 60$ minutes. In addition, we find that the additional effect of proteins on the CHO-induced insulin response as described previously [27,29] is not influenced by the timing of consumption of the protein or carbohydrate load. This can be deducted from the observation that protein hydrolysate consumption with CHO both as pre- and afterload induces insulin responses similar to each other, which are double the insulin response after a single protein or CHO load.

Furthermore, we found that when SPH consumption was followed by an equi-energetic CHO load, this CHO consumption did not result in a rise in plasma glucose concentrations. This suggests that in this condition, glucose that reaches circulation disappears rapidly into tissues to be metabolized. This can be explained by the already elevated plasma insulin levels induced by the protein load before CHO consumption. The rise in lactate when the CHO is ingested after the protein suggests that glucose is partly metabolized into lactate in an extrahepatic way, which is in accordance with the glucose paradox [30]. The finding that protein ingestion before CHO prevents the CHO-induced increase in blood glucose is of interest in view of the discussion about glycemic index and its effect on health [31] because this condition may allow ingestion of high–glycemic index carbohydrates without the associated high blood glucose response. To find out how protein/peptide preloads influence plasma glucose levels over 24 hours or over longer periods, long-term studies with high-protein diets should be performed.

Concerning glucagon responses, no differences were found in the overall response induced by SPH with a CHO pre- or afterload. Fig. 4, however, shows that the pattern of the glucagon responses is clearly different. Although postprandial insulin-driven suppression of glucagon secretion has been suggested [32], we did not find an effect of the CHO preload–induced insulin response on the glucagon-inducing effect of SPH. This observation supports the hypothesis that the amino acid–induced glucagon secretion is not or only partly inhibited by increased insulin levels [33].

4.3. Soy vs PPH with CHO afterload

In this additional trial, we wanted to study the effect of a different vegetable protein source (pea protein) of which a previous study showed that it induced the highest insulin and glucagon levels compared with the other protein hydrolysates from vegetable origin tested (soy, rice, and gluten) (manuscript under review).

No significant differences in DIT or RQ were found between both conditions, but PPH induced significantly higher insulin and glucagon responses compared to SPH. Although PPH induced a higher glucagon response, we did not find significantly higher DIT compared to SPH. This may be because the absolute difference in glucagon concentration at the different time points was not large enough for inducing higher EE. Another explanation could be that the effect of glucagon on DIT was already maximal at the glucagon concentration attained after SPH consumption and an additional increase in glucagon concentration could not increase DIT further.

The explanation for the higher insulin and glucagon responses after PPH consumption than after SPH consumption is not directly evident from our study. Calbet and MacLean [9] reported previously that the rate of gastric emptying of pea and whey hydrolysate was comparable. Although we cannot exclude the possibility that SPH has a different rate of gastric emptying than whey protein hydrolysate, this does not seem very likely. The difference in insulin and glucagon responses between both protein sources can, therefore, not be explained by differences in gastric emptying. In a previous study we found that the branched-chain amino acids (leucine, isoleucine, and valine) were the best predictors of the insulin and glucagon responses (manuscript under review).

Because soy and pea protein contain similar amounts of branched-chain amino acids, differences in branched-chain amino acid content of SPH and PPH cannot explain the difference in glucagon and insulin responses. Specific amino acids, such as arginine, glycine, alanine, lysine, phenylalanine, methionine, and tyrosine, have been reported to be more insulin- and/or glucagon-stimulating than others [9,34-37]. Because arginine and lysine were more abundant in PPH than in SPH, this difference might be responsible for the differences found in insulin and glucagon responses between SPH and PPH.

4.4. Association between insulin and/or glucagon and EE

To find out to what extent insulin and glucagon separately and together contribute to changes in EE independent of the trials, we performed multiple linear regression analyses corrected for subject, trial and, time point with EE as dependent variable and glucagon and/or insulin concentrations as independent variable. These analyses showed that insulin contributes most to changes in EE (27.9%), but when glucagon is also included in the model, significantly more variation in EE is explained (33.2%, $P < .001$). Whether insulin and glucagon have a direct effect on EE remains to be investigated.

In summary, a single protein hydrolysate drink induced a more prolonged increase in EE, similar insulin response, and higher glucagon response than an equi-energetic carbohydrate drink. Plasma insulin and glucagon were both predictors of EE. The rise in plasma glucose after carbohydrate consumption did not occur when ingestion of the carbohydrate drink was preceded by ingestion of a protein hydrolysate drink. Finally, it is likely that there will be no large differences in response after ingestion of intact protein drinks compared to ingestion of hydrolysates [9], but the effect of ingesting high-protein and/or high-carbohydrate foods remains to be investigated.

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