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Differential effects of proteins and carbohydrates on postprandial blood pressure-related responses

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Abstract

Diet composition may affect blood pressure (BP), but the mechanisms are unclear. The aim of the present study was to compare postprandial BP-related responses to the ingestion of pea protein, milk protein and egg-white protein. In addition, postprandial BP-related responses to the ingestion of maltodextrin were compared with those to the ingestion of sucrose and a protein mix. We hypothesised that lower postprandial total peripheral resistance (TPR) and BP levels would be accompanied by higher plasma concentrations of nitric oxide, insulin, glucagon-like peptide 1 (GLP-1) and glucagon. On separate occasions, six meals were tested in a randomised order in forty-eight overweight or obese adults with untreated elevated BP. Postprandial responses of TPR, BP and plasma concentrations of insulin, glucagon, GLP-1 and nitrite, nitroso compounds (RXNO) and S-nitrosothiols (NO_x) were measured for 4 h. No differences were observed in TPR responses. Postprandial BP levels were higher after the ingestion of the egg-white-protein meal than after that of meals containing the other two proteins ($P \leq 0{\cdot}01$). The ingestion of the pea-protein meal induced the highest NO_x response ($P \leq 0{\cdot}006$). Insulin and glucagon concentrations were lowest after the ingestion of the egg-white-protein meal ($P \leq 0{\cdot}009$). Postprandial BP levels were lower after the ingestion of the maltodextrin meal than after that of the protein mix and sucrose meals ($P \leq 0{\cdot}004$), while postprandial insulin concentrations were higher after the ingestion of the maltodextrin meal than after that of the sucrose and protein mix meals after 1–2 h ($P \leq 0{\cdot}0001$). Postprandial NO_x, GLP-1 and glucagon concentrations were lower after the ingestion of the maltodextrin meal than after that of the protein mix meal ($P \leq 0{\cdot}008$). In conclusion, different protein and carbohydrate sources induce different postprandial BP-related responses, which may be important for BP management. Lower postprandial BP levels are not necessarily accompanied by higher NO_x, insulin, glucagon or GLP-1 responses.

Key words: **Protein sources: Carbohydrates: Blood pressure**

The effect of dietary proteins on blood pressure (BP) has been evaluated in two recent meta-analyses, which concluded that replacement of part of dietary carbohydrates with proteins is beneficial in BP management[1,2]. It is still unclear whether different dietary proteins have different effects on BP[3]. Randomised trials comparing the effects of prolonged intake of different types of proteins or different amino acids on BP are scarce. Mainly, soya and milk proteins have been studied in this context, and a recent meta-analysis has shown that soya and milk proteins decrease BP to a similar extent[4]. The postprandial BP responses to the ingestion of the milk proteins casein and whey have been found to be similar to those to the ingestion of a carbohydrate control[5]. In a randomised clinical trial on the effects of PROteins on blood PRESsure

Abbreviations: ACE, angiotensin-converting enzyme; AIx, augmentation index; BP, blood pressure; CO, cardiac output; DBP, diastolic blood pressure; GLP-1, glucagon-like peptide 1; HR, heart rate; iAUC, incremental AUC; MAP, mean arterial pressure; NO_x, nitrite, nitroso compounds (RXNO) and S-nitrosothiols; PP, pulse pressure; PROPRES, randomised clinical trial on the effects of PROteins on blood PRESsure; PWV, pulse wave velocity; SBP, systolic blood pressure; TPR, total peripheral resistance.

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(PROPRES), we found that the consumption of 60 g/d of a protein mix for 4 weeks lowered BP compared with that of 60 g/d of maltodextrin[6]. Additional research in a subgroup of the PROPRES study has shown that the acute BP responses to the ingestion of mixed meals supplemented with the protein mix or maltodextrin differed. We found a smaller postprandial decrease in BP after the ingestion of a protein-supplemented meal than after that of a maltodextrin-supplemented meal, and we suggested that differences in insulin responses might explain these differences in the postprandial BP responses[7]. From this study, it remained unclear whether the differences in BP responses after carbohydrate and protein intake can be generalised to ingestion of any kind of proteins and carbohydrates or are dependent on the type of protein or carbohydrate ingested. The aim of the present study was to investigate postprandial BP responses to the ingestion of proteins and carbohydrates from different sources. An additional objective was to explore the mechanisms via which different dietary factors affect BP. Postprandial responses are of interest because in clinical practice BP is often measured in the postprandial state and may therefore be influenced by prior meal ingestion. Moreover, prognostic epidemiological data are usually based on BP measurements taken during daily clinical practice, which are mostly not recorded in the fasted state. We hypothesised that lower postprandial total peripheral resistance (TPR) and BP levels would be accompanied by higher postprandial concentrations of plasma nitric oxide (NO). NO is a potent vasodilator produced in the vascular endothelium. Several hormones, such as insulin and glucagon-like peptide-1 (GLP-1), and amino acids, such as arginine, may increase endothelial NO release[8–10]. The concentrations of these hormones and amino acids may be affected by protein ingestion[11–14]. Therefore, our second hypothesis was that higher postprandial NO concentrations would be accompanied by higher concentrations of insulin, GLP-1 and/or glucagon. Glucagon has been hypothesised to increase NO production because of its vasodilatory effects; however, these effects can differ between vascular beds[15] and the effects of glucagon on NO release may be dependent on the condition studied[16,17]. In the present study, the postprandial effects of pea, milk and egg-white protein isolates were compared. These proteins were selected because they were included in the protein mix that lowered BP in the PROPRES study[6]. In addition, we investigated whether the choice of our carbohydrate control in the PROPRES study[6] could have influenced our previous findings. Most studies on the BP-lowering effect of proteins have compared the effects of dietary proteins with those of carbohydrates[18]. However, BP may respond differently after the consumption of different carbohydrate sources[19]. Therefore, we chose to compare the postprandial effects of maltodextrin with those of sucrose, a widely consumed carbohydrate (table sugar) with a less pronounced insulin response compared with maltodextrin. We hypothesised that TPR and BP would decrease more after the consumption of maltodextrin than after that of sucrose due to a greater insulin response. Responses to the ingestion of maltodextrin were also compared with those to the ingestion of the protein mix tested in the PROPRES study[6].

Subjects and methods

Subjects

Subjects were recruited through local newspapers and via our database of subjects of the PROPRES study[6]. Inclusion and exclusion criteria for the determination of subject eligibility were similar to those of the PROPRES study[6]. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the medical ethics committee of Maastricht University Medical Center and Maastricht University (METC azM/UM). Written informed consent was obtained from all the subjects. The study was conducted at Maastricht University (Netherlands) between January and December 2011.

Study diets and design

The present study was a double-blind, six-arm randomised cross-over trial. If eligible, the subjects were enrolled for a run-in period of 2 weeks, during which they followed standard dietary advice as described previously[6]. If their BP still met the inclusion criteria after the 2-week run-in period, the subjects were randomised to one of the six treatment orders using a computer program (MINIM; Stephen Evans, Simon Day and Patrick Royston; http://www-users.york.ac.uk/~mb55/guide/minim.htm). The subjects followed the dietary advice until the end of the study. This trial was registered at http://www.trialregister.nl: as NTR2678.

Test meals were six different powders containing either a protein or a carbohydrate mixed with H_2O at a ratio of 1:4 and were consumed on six separate test days with a washout period of 1 week between each test. The test meals consisted of 0·6 g of protein or carbohydrate per kg body mass. Protein products tested were a pea protein isolate (Roquette), a milk protein isolate (DMV International), an egg-white protein isolate (Noventum Foods), and a mix of protein isolates consisting of 20 % pea protein, 20 % soya protein (ADM Specialty Food Ingredients), 30 % milk protein and 30 % egg-white protein. Carbohydrate products tested were maltodextrin (Syral) and sucrose (Suiker Unie). All the test products were of food grade. The test powders were matched for fat and mineral content (Table 1) and mixed by NIZO food research. The amino acid compositions of the protein isolates are given in Table 2. The researchers and subjects were blinded to the meals. On the day before each test day, all the subjects consumed the same foods, which were provided by the researchers (15 % energy from protein, 30 % energy from fat and 55 % energy from carbohydrate). On the test day, the subjects arrived at the university at 08.00 hours after an overnight fast. The test meals were consumed at T0. Measurements were taken at time points −1, + 1, + 2, + 3 and + 4 h.

Measurements

During the screening visit and run-in period, BP and heart rate (HR) measurements were taken as described previously[6]. On the test day, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and HR were measured using a Spacelabs 90 207 (Spacelabs Healthcare

Table 1. Meal compositions for 70 g of protein or carbohydrate

	Milk protein	Egg-white protein	Pea protein	Protein mix	Maltodextrin	Sucrose
Energy (kJ)	1481	1443	1506	1460	1439	1460
Protein (g)	70	70	70	70	0·37	0·36
Carbohydrates (g)	2·8	2·6	2·1	1·7	70	70
Fat (g)	7	6	8	6·9	7	7·5
Fibre (g)	0·9	3·7	1·6	1·9	3·6	1·6
Minerals						
Na (mg)	940	980	1010	1060	1050	910
K (mg)	830	700	940	830	710	820
Ca (mg)	1170	1060	1160	1160	1120	1110
P (mg)	900	820	920	910	910	860
Mg (mg)	66	67	73	71	69	62

Limited), a validated automated BP monitor[20], with the subjects sitting on a bed in a semi-supine position. Cardiac output (CO) and stroke volume were measured non-invasively using the Finometer MIDI and BeatScope Easy software (Finapres Medical Systems)[21]. TPR was calculated from the MAP and CO as described previously[7]. Pulse pressure (PP) was calculated as follows: SBP − DBP. The central augmented pressure:pulse height ratio (augmentation index (AIx)) was derived from pulse wave analysis at the arteria radialis using the SphygmoCor CP system (Atcor Medical) and corrected for a HR of 75 beats/min. Pulse wave velocity (PWV) was measured using the Vicorder (Skidmore Medical Limited). PWV measurements were taken with one loose cuff around the neck monitoring pulse waves in the arteria carotis and another cuff around the upper leg monitoring pulse waves in the arteria femoralis. PWV was assessed by recording the distance between the sternal notch and the middle of the leg cuff and dividing this by the time lag between the pulses from the carotis and femoralis. PWV was measured three times, and the average was used in analyses.

Serum and plasma analyses

Blood samples were collected in tubes containing heparin and lithium for the determination of nitrite, nitroso compounds (RXNO) and S-nitrosothiols (NO_x). The samples in the tubes were centrifuged within 10 min of collection for 17 min at 4°C and 87 ***g***. Plasma was collected and stored at −80°C until analysis. The concentrations of NO_x were determined using a previously described chemiluminescence technique of Rikilt[22]. The concentrations of plasma glucose, GLP-1, glucagon, angiotensin-converting enzyme (ACE) and serum insulin were measured by MLM Medical Labs. The concentrations of plasma glucose and serum insulin were determined as described previously[7]. Blood samples were collected in EDTA collection tubes containing a dipeptidyl peptidase-IV inhibitor for GLP-1 analysis. The concentrations of active GLP-1 were measured with an ELISA (Linco Research). Blood samples were collected in EDTA tubes containing aprotinin for glucagon analysis. The concentrations of glucagon were determined using a RIA (Euro-Diagnostic). The activity of ACE was also measured, because dietary proteins can affect BP via the inhibition of ACE[23]. The activity of ACE was determined using the ACE colour method (Fujirebio).

Table 2. Amino acid compositions (g/100 g) of the protein sources

	Milk protein	Egg-white protein	Pea protein
Ala	2·9	4·3	4·2
Arg	3·7	6	8·7
Asp	6·7	9·6	11·5
Cys	0·6	3	1·1
Glu	19·5	17	17·2
Gly	1·6	3·2	4·2
His	2·5	2·5	2·5
Ile	4·7	4·9	4·8
Leu	8·8	8·4	8·3
Lys	7·5	6·8	7·3
Met	2·6	2·3	1
Phe	4·4	5·1	5·3
Pro	9·7	6	4·5
Ser	5·1	5·6	5·1
Thr	3·9	4	4
Trp	1·1	1·2	1
Tyr	5	4·2	3·8
Val	6·3	5·8	5

Statistical analyses

Baseline characteristics are reported as means with their standard errors. Changes in weight between week 3 (end of the run-in period) and week 8 were tested using a paired *t* test. Postprandial responses were analysed with a linear mixed model approach to take the cross-over design and the correlation between repeated measures into account. The basic model consisted of a random intercept at the individual level. If significant, this model was extended with a random intercept at the meal within the individual level (individual × meal) or with a serial correlation over time. The basic model always included the following variables: time; meal; baseline measurements; two variables controlling for the cross-over design: meal order and test day number. The interaction term between meal and time (meal × time) and the covariates age, sex and BMI were included in the model if $P \leq 0{\cdot}05$. In case of a significant meal × time or meal effect, five *post hoc* comparisons were made, i.e. maltodextrin *v.* sucrose, maltodextrin *v.* protein mix, pea protein *v.* milk protein, pea protein *v.* egg-white protein, and milk protein *v.* egg-white protein. The critical *P* value was corrected for these comparisons; therefore; *post hoc* differences were considered significant at $P \leq 0{\cdot}01$. To determine whether postprandial changes from baseline were significant, the incremental AUC (iAUC) was tested for a significant difference from zero for each meal.

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This was done using a one-sample *t* test and a critical *P* value corrected for the six meals ($P=0{\cdot}0083$). All the analyses were carried out using SPSS software (version 19; IBM). The present study was powered to show a significant difference in TPR of at least 1 mmHg/l per min with a SD of 2·2 mmHg/l per min with a power of 0·8. Based on these values, forty participants would be needed. The aim was to include fifty participants, accounting for a dropout rate of 20 %.

Results

Subjects

A total of forty-eight subjects were included in the present study (Table 3). The subjects lost an average of 0·4 (SEM 0·2) kg of body mass during the six test weeks ($P=0{\cdot}009$). The carbohydrate test meals were well tolerated, but some subjects had trouble with fully finishing the protein meals. Of these subjects, three were unable to finish the pea-protein meal, three were unable to finish the milk-protein meal, one subject did not finish the egg-white-protein meal and one subject did not finish the protein mix meal completely. Because most of the meal was still consumed in these cases, data obtained from these subjects were included in all the analyses.

Postprandial responses

Overall, significant time × meal interactions were found for SBP, DBP, MAP, HR, AIx, NO_x, glucose, insulin, GLP-1 and glucagon responses after the ingestion of the six meals ($P \leq 0{\cdot}03$). PP responses to the ingestion of the six meals differed significantly, independent of time ($P = 0{\cdot}007$). Details on how responses to the ingestion of the six meals differed are discussed below. TPR, CO, stroke volume, PWV and ACE responses to the ingestion of the six meals did not differ significantly.

Responses to the ingestion of egg-white, milk and pea proteins

The ingestion of the egg-white-protein meal resulted in a significant increase in SBP, MAP and PP, while that of the pea-protein meal significantly reduced DBP (iAUC, $P\leq0{\cdot}001$). At 2–4 h, MAP was significantly higher after the ingestion of the egg-white-protein meal than after that of meals containing the other two proteins ($P\leq0{\cdot}01$). SBP and DBP were also significantly higher after the ingestion of the egg-white-protein meal than after that of meals containing the other two proteins at most time points ($P\leq0{\cdot}008$; Fig. 1(a)–(c)). PP was significantly higher after the ingestion of the egg-white-protein meal than after that of the milk-protein meal, independent of time ($P=0{\cdot}009$, data not shown). The ingestion of the pea-protein meal significantly decreased TPR and increased HR, while only the ingestion of the milk-protein meal significantly increased CO (iAUC, $P\leq0{\cdot}005$; Fig. 1(d)–(f)). Postprandial HR was significantly higher after the ingestion of the pea-protein meal than after that of the egg-white-protein meal between 1 and 3 h ($P\leq0{\cdot}008$; Fig. 1(d)). The ingestion of all the three protein meals resulted in a decrease in the AIx (iAUC, $P\leq0{\cdot}001$). The AIx was significantly higher after the ingestion of the egg-white-protein meal than after that of the pea-protein meal at 1 h and higher than that after the ingestion of the milk-protein meal at 3 h ($P\leq0{\cdot}002$; Fig. 1(g)).

Plasma NO_x concentrations were significantly increased after the ingestion of the pea- and milk-protein meals (iAUC, $P\leq0{\cdot}0001$). NO_x concentrations were significantly higher after the ingestion of the pea-protein meal than after that of meals containing the other two proteins at 1–3 h ($P\leq0{\cdot}006$; Fig. 1(h)). The ingestion of all the three protein meals significantly reduced plasma glucose concentrations and increased insulin concentrations (iAUC, $P\leq0{\cdot}0001$; Fig. 1(i) and (j)). No differences were observed in the postprandial plasma glucose responses to the ingestion of all the three protein meals (Fig. 1(i)). Serum insulin concentrations were significantly lower at 1–3 h after the ingestion of the egg-white-protein meal than after that of the milk-protein meal and at 1–2 h compared with those after the ingestion of the pea-protein meal ($P\leq0{\cdot}0001$). Serum insulin concentrations were significantly higher after the ingestion of the milk-protein meal than after that of the pea-protein meal after 4 h ($P=0{\cdot}009$; Fig. 1(j)). Postprandial plasma glucagon concentrations were increased after the ingestion of all the three protein meals (iAUC, $P\leq0{\cdot}0001$; Fig. 1(k)), but remained lowest after the ingestion of the egg-white-protein meal than after that of meals containing the other two proteins at 1–3 h ($P\leq0{\cdot}009$). At 1–2 h, plasma glucagon concentrations observed after the ingestion of the pea-protein meal also differed significantly from those observed after the ingestion of the milk-protein diet, with the highest plasma glucagon concentrations being detected after the ingestion of the pea-protein meal ($P\leq0{\cdot}003$; Fig. 1(k)). Plasma GLP-1 concentrations were increased after the ingestion of all the three protein meals (iAUC, $P\leq0{\cdot}0001$; Fig. 1(l)), but were significantly lower after the ingestion of the egg-white-protein meal than after that of meals containing the other two proteins after 2 h ($P\leq0{\cdot}0001$) while being higher after the ingestion of the egg-white-protein meal than after that of the pea-protein meal after 4 h ($P=0{\cdot}002$; Fig. 1(l)). Plasma ACE activity was significantly decreased after the ingestion of the pea-protein meal (iAUC, $P=0{\cdot}005$, data not shown); however, no differences were observed in the plasma ACE activity responses to the ingestion of the three protein meals.

Table 3. Baseline characteristics of the study participants
(Mean values with their standard errors)

	Mean	SEM
Sex (*n*)		
Male	31	
Female	17	
Age (years)	58	1
BMI (kg/m^2)	28·6	0·3
Fasting plasma glucose (mmol/l)	5·5	0·1
eGFR* (ml/min per 1·73 m^2)	99	3
SBP during the run-in period (mmHg)	144	1
DBP during the run-in period (mmHg)	92	1

eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Estimated by the modification of the diet in renal disease formula.

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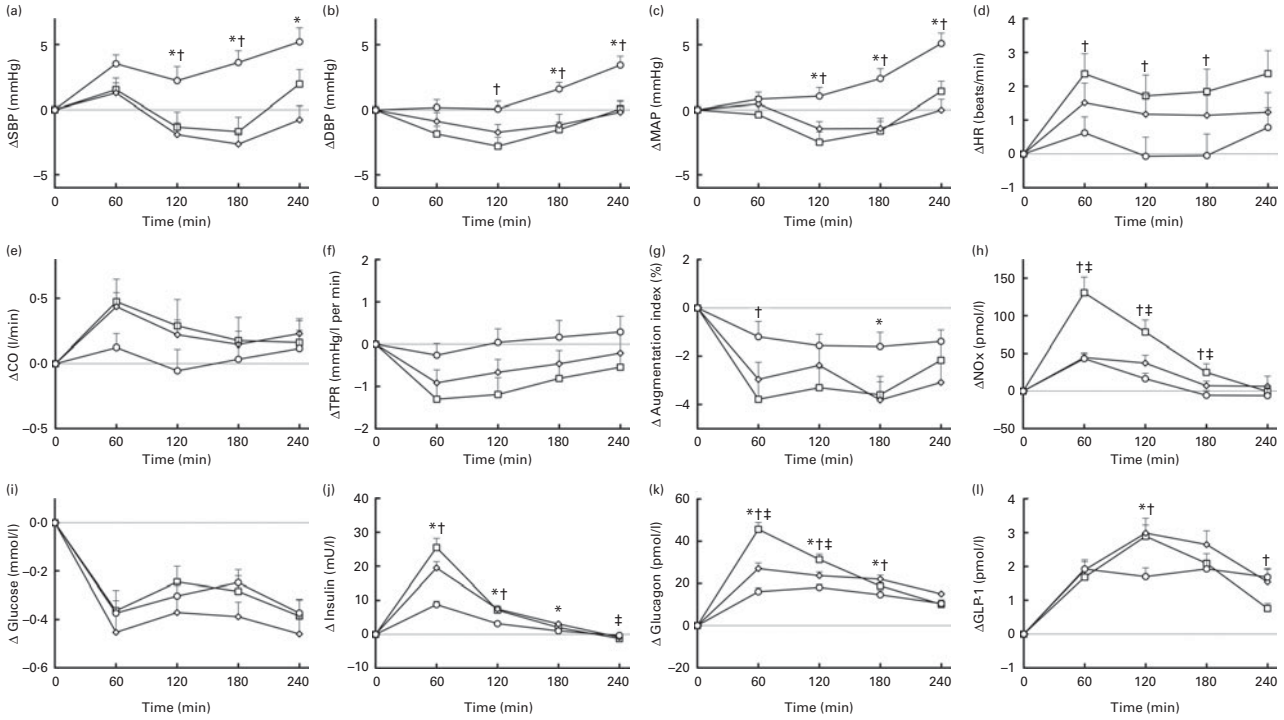

Fig. 1. Changes in (a) systolic blood pressure (SBP), (b) diastolic blood pressure (DBP), (c) mean arterial pressure (MAP), (d) heart rate (HR), (e) cardiac output (CO), (f) total peripheral resistance (TPR), (g) augmentation index, (h) nitrite, nitroso compound and *S*-nitrosothiol (NO_x), (i) glucose, (j) insulin, (k) glucagon and (l) glucagon-like-peptide 1 (GLP-1) responses. Values are means, with their standard errors represented by vertical bars. For SBP, DBP, MAP, HR, augmentation index, NO_x, glucose, insulin and glucagon: *n* 47 for pea protein and egg-white protein; *n* 48 for milk protein. For CO and TPR: *n* 47 for pea protein and egg-white protein; *n* 46 for milk protein. For GLP-1: *n* 47 for pea protein; *n* 48 for milk protein; *n* 45 for egg-white protein. *, †, ‡ Significant differences between protein sources (□, pea protein; ◇, milk protein; ○, egg-white protein) shown by *post hoc* tests with Bonferroni correction ($P \leq 0{\cdot}01$) if the time × meal interaction was significant ($P \leq 0{\cdot}05$; linear mixed model). * Comparison of egg-white protein *v.* milk protein. † Comparison of egg-white protein *v.* pea protein. ‡ Comparison of milk protein *v.* pea protein.

Responses to the ingestion of maltodextrin compared with those to the ingestion of the protein mix and sucrose

DBP and MAP were significantly decreased after the ingestion of the maltodextrin meal (iAUC, $P \leq 0{\cdot}0004$), but not after that of the protein mix or sucrose meal. SBP was not significantly increased or decreased after the ingestion of any of the three meals. PP was significantly increased after the ingestion of the protein mix meal (iAUC, $P = 0{\cdot}0001$, data not shown). SBP, DBP and MAP were significantly lower at 1 h after the ingestion of the maltodextrin meal than after that of the sucrose and protein mix meals ($P \leq 0{\cdot}003$). DBP and MAP were also significantly lower after the ingestion of the maltodextrin meal than after that of the sucrose meal after 2 h ($P \leq 0{\cdot}004$; Fig. 2(a)–(c)). TPR was significantly decreased after the ingestion of the protein mix meal (iAUC, $P = 0{\cdot}0001$; Fig. 2(f)), while HR and CO were significantly increased after the ingestion of this meal (iAUC, $P \leq 0{\cdot}001$; Fig. 2(d) and (e)). TPR, CO and HR did not change significantly after the ingestion of the maltodextrin or sucrose meal. Postprandial HR was significantly higher after the ingestion of the protein mix meal than after that of the maltodextrin meal after 2–4 h ($P \leq 0{\cdot}0001$; Fig. 2(d)). The AIx was significantly decreased after the ingestion of the maltodextrin and protein mix meals (iAUC, $P \leq 0{\cdot}0001$), but not after that of the sucrose meal. The AIx was significantly higher at 2 h after the ingestion of the sucrose meal than after that of the maltodextrin meal ($P = 0{\cdot}005$; Fig. 2(g)).

Plasma NO_x concentrations were significantly increased after the ingestion of the protein mix meal (iAUC, $P \leq 0{\cdot}0001$). NO_x concentrations were significantly higher at 1–2 h after the ingestion of the protein mix meal than after that of the maltodextrin meal ($P \leq 0{\cdot}008$), but were lower at 2 h after the ingestion of the sucrose meal than after that of the maltodextrin meal ($P = 0{\cdot}002$; Fig. 2(h)). The ingestion of the maltodextrin meal resulted in significant increases in glucose and insulin concentrations (iAUC, $P \leq 0{\cdot}0001$). The ingestion of the sucrose and protein mix meals also increased insulin concentrations (iAUC, $P \leq 0{\cdot}0001$). In addition, the ingestion of the protein mix meal significantly lowered plasma glucose concentrations (iAUC, $P \leq 0{\cdot}0001$; Fig. 2(i)–(j)). The ingestion the maltodextrin meal resulted in significantly higher plasma glucose concentrations at 1–2 h and serum insulin concentrations at 1–3 h compared with that of the sucrose meal ($P \leq 0{\cdot}002$). Compared with those observed after the ingestion of the protein mix meal, plasma glucose and serum insulin concentrations were significantly higher at 1–2 h after the ingestion of the maltodextrin meal and significantly lower at 3–4 h after that of the maltodextrin meal ($P \leq 0{\cdot}0001$). Plasma glucagon concentrations were significantly decreased after the ingestion of the maltodextrin and sucrose meals and significantly increased after

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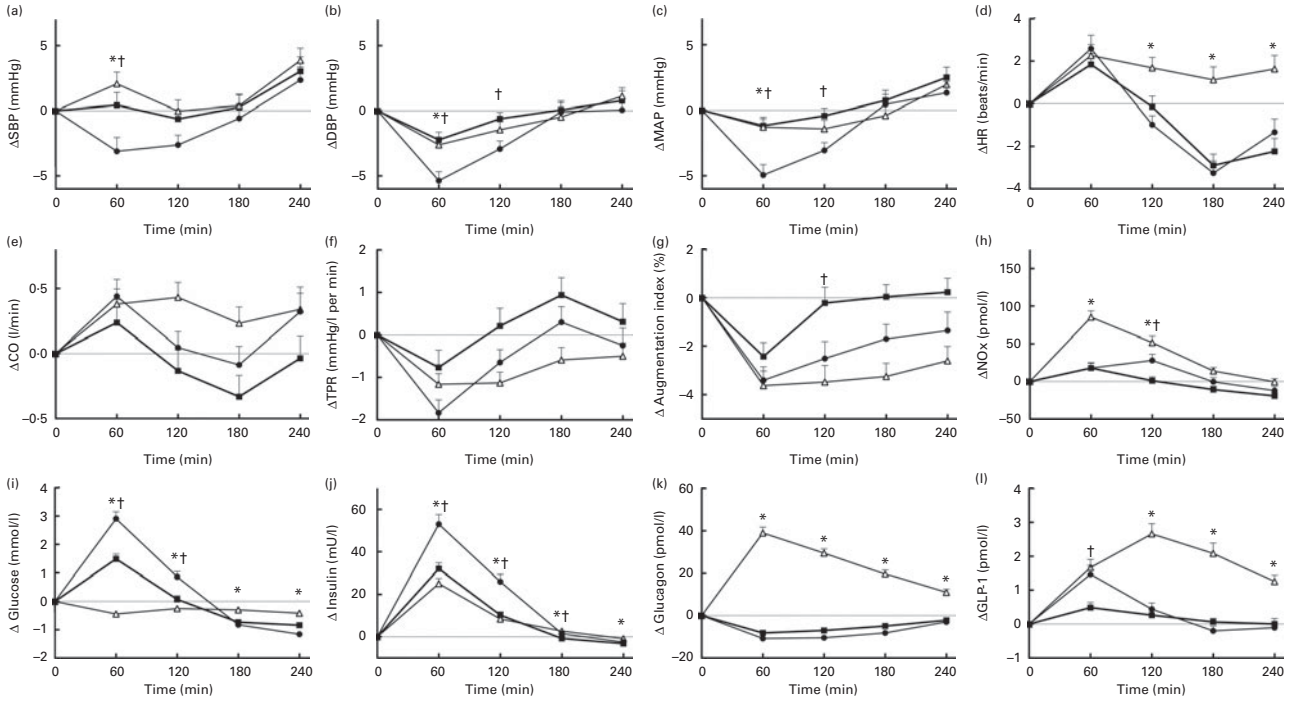

Fig. 2. Changes in (a) systolic blood pressure (SBP), (b) diastolic blood pressure (DBP), (c) mean arterial pressure (MAP), (d) heart rate (HR), (e) cardiac output (CO), (f) total peripheral resistance (TPR), (g) augmentation index, (h) nitrite, nitroso compound and *S*-nitrosothiol (NO_x), (i) glucose, (j) insulin, (k) glucagon and (l) glucagon-like-peptide 1 (GLP-1) responses. Values are means, with their standard errors represented by vertical bars. For SBP, DBP, MAP, HR, augmentation index, glucose, insulin and glucagon: *n* 47 for maltodextrin and sucrose; *n* 48 for the protein mix. For CO, TPR, glucose, insulin, glucagon and GLP-1: *n* 47 for maltodextrin; *n* 48 for the protein mix; *n* 46 for sucrose. For NO_x: *n* 46 for maltodextrin and sucrose; *n* 48 for the protein mix. * Significant differences between maltodextrin (●) and the protein mix (Δ) shown by *post hoc* tests with Bonferroni correction ($P \leq 0{\cdot}01$) if the time × meal interaction was significant ($P \leq 0{\cdot}05$; linear mixed model). † Significant differences between maltodextrin and sucrose (■) shown by *post hoc* tests with Bonferroni correction ($P \leq 0{\cdot}01$) if the time × meal interaction was significant ($P \leq 0{\cdot}05$; linear mixed model).

the ingestion of the protein mix meal (iAUC, $P \leq 0{\cdot}0001$; Fig. 2(k)). Glucagon concentrations observed after the ingestion of the maltodextrin meal did not differ from those observed after the ingestion of the sucrose meal. Glucagon concentrations were significantly higher after the ingestion of the protein mix meal than after that of the maltodextrin meal at 1–4 h ($P \leq 0{\cdot}0001$). GLP-1 concentrations were significantly increased after the ingestion of the maltodextrin and protein mix meals (iAUC, $P \leq 0{\cdot}0001$; Fig. 2(l)). GLP-1 concentrations were significantly higher at 1 h after the ingestion of the maltodextrin meal than after that of the sucrose meal ($P \leq 0{\cdot}0001$). After 2–4 h, GLP-1 concentrations were significantly higher after the ingestion of the protein mix meal than after that of the maltodextrin meal ($P \leq 0{\cdot}0001$; Fig. 2(l)). ACE activity was significantly decreased after the ingestion of the maltodextrin and protein mix meals ($P \leq 0{\cdot}001$, data not shown); however, there were no differences in the postprandial ACE activity responses to the ingestion of the maltodextrin, sucrose and protein mix meals.

Discussion

In the present study, we compared the acute postprandial BP-related responses to the ingestion of egg-white, milk and pea proteins and we compared postprandial BP-related responses to the ingestion of maltodextrin with those to the ingestion of sucrose and a protein mix.

When comparing the protein sources, egg-white protein was found to induce the highest postprandial BP levels compared with the pea and milk proteins. We hypothesised that NO-induced vasodilation might be the mechanism responsible for lowering TPR and consequently BP. The higher BP levels and lower NO_x response observed after the ingestion of the egg-white-protein meal compared with the levels observed after the ingestion of the pea-protein meal are in agreement with this hypothesis, but the BP levels following the ingestion of the milk-protein meal were lower than those following the ingestion of the egg-white-protein meal, despite a similar NO_x response. An additional mechanism, such as ACE inhibition, might have influenced the BP response after the ingestion of the milk-protein meal[23], but we did not find significant differences in the postprandial plasma ACE activity responses to the ingestion of the protein meals. In addition, we found no differences in TPR or CO responses to the ingestion of the protein sources. We also hypothesised that NO could be induced by insulin, glucagon and GLP-1[9,10,15]. In accordance with this, pea protein induced the highest hormonal and NO_x responses, while egg-white protein induced the lowest responses. The higher amount of arginine in pea protein could have also contributed to the higher NO_x response[8]. However, milk protein induced a low NO_x response, despite inducing intermediate hormonal responses. NO regulation is complex and is influenced by many pathways. For instance, leptin is also known to induce NO[24], while glucagon can counteract

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insulin-induced NO release[16]. In addition, several proteins and amino acids have been reported to affect NO synthesis in a variety of tissues[25].

The AIx was decreased after the ingestion of all the three protein meals, which indicates that the diameter or distensibility of arteries or arterioles was increased[26]. A postprandial decrease in AIx has been reported previously[27]. The smaller decrease in AIx after the ingestion of the egg-white-protein meal could be due to the lower insulin response. Westerbacka *et al.*[26] demonstrated that a physiological dose of insulin can decrease AIx within 1 h.

Differences in insulin- and glucagon-stimulating properties of different proteins have been reported previously[11,13,28] and may be explained by differences in the amino acid composition. Phenylalanine and glycine, which are present in highest amounts in pea protein, have been found to be more insulinotropic compared with other amino acids[12]. Glycine and arginine, also most abundant in pea protein, have been found to be have higher glucagon-stimulating properties[12]. Branched-chain amino acids, which are more common in milk proteins, have also been reported to have higher insulin-stimulating properties[11]. Differences in the rate of digestion can also influence postprandial insulin responses as shown in studies comparing insulin responses to the consumption of the fast protein whey and the slow protein casein[29,30]. However, the relative rates of digestion of the proteins in the present study are unknown. GLP-1 responses to the ingestion of the different protein sources did not seem to differ much. Limited and conflicting data have been reported in the literature on the effects of different proteins on GLP-1 concentrations. No differences were found in GLP-1 concentrations by two studies after the consumption of meals containing 18 g of milk, cheese, whey, cod or wheat gluten protein combined with 25 g of carbohydrates[31] or after the consumption of different amino acid mixtures containing isoleucine, leucine, valine and/or threonine, lysine and whey protein supplemented with 25 g carbohydrates[32]. A higher GLP-1 response was found by one study after the consumption of whey protein than after that of casein[14]. Thus, as hypothesised, the lower BP levels observed after the ingestion of the pea-protein meal compared with the levels observed after the ingestion of the egg-white-protein meal was accompanied by higher NO_x, insulin, glucagon and GLP-1 responses. However, the lower BP response and higher insulin, glucagon and GLP-1 responses observed after the ingestion of the milk-protein meal compared with the responses observed after the ingestion of the egg-white-protein meal were not accompanied by higher plasma NO_x concentrations. Therefore, lower postprandial BP levels and higher concentrations of serum insulin and plasma glucagon and GLP-1 are not always accompanied by higher plasma NO_x concentrations.

When comparing maltodextrin and sucrose, we found significantly lower BP levels after the ingestion of the maltodextrin meal. It has previously been suggested that this may be due to the fructose content of sucrose, but a recent meta-analysis has found no significant effect of prolonged fructose intake on BP in human trials[33]. We hypothesised that the lower BP levels could be due to a higher NO_x response. Indeed, we found significantly higher NO_x concentrations after the ingestion of the maltodextrin meal, but only at 2 h. We did not find significant changes in TPR, CO and HR after the ingestion of either carbohydrate. An acute study in healthy subjects found a higher BP increase after fructose consumption than after glucose consumption[19]. In contrast to the present study, this study also reported a greater decrease in TPR and a greater increase in CO after glucose consumption[19]. A study in healthy elderly subjects (65–78 years) reported that the decrease in SBP, DBP and MAP was similar 1 h after the consumption of a beverage containing 50 g glucose compared with that observed after the consumption of a beverage containing sucrose[34]. Another study comparing a glucose drink with a glucose–fructose drink (45 g glucose and 55 g fructose) only found a higher HR after the consumption of the glucose–fructose drink, with no differences being detected in BP, PWV and nitrite:nitrate in 90 min AUC[35]. The higher insulin and GLP-1 responses induced by the higher glucose content of maltodextrin in combination with the higher NO_x response compared with the responses observed after the ingestion of the sucrose meal support our second hypothesis that NO_x may be induced by these hormones. Others have also reported higher insulin responses after the consumption of glucose than after that of fructose[36–38], while GLP-1 concentrations have been found either to be higher[36] or to not differ[38]. However, the time points at which the differences in insulin and GLP-1 responses were observed in the present study were not completely in agreement with our hypothesis, as the concentrations of both hormones were highest after 1 h, while NO_x concentrations were higher at 2 h. The AIx was significantly lower after the ingestion of the maltodextrin meal than after that of the sucrose meal at 2 h, which may be due to the higher insulin response[26].

When comparing BP responses to the ingestion of the protein mix and maltodextrin, we did find lower BP levels at 1 h after the ingestion of the maltodextrin meal, as we found in the PROPRES study[7]. In contrast to the PROPRES study, in which both the carbohydrate and protein meals induced a decrease in BP, the protein meals induced no change or an increase in BP in the present study. However, in the present study, proteins and maltodextrin were tested separately, while in the PROPRES study both were consumed with a mixed meal[7]. We hypothesised that lower postprandial BP levels would be accompanied by a higher NO_x concentration, which decreases TPR by inducing vasodilation. The NO_x response was higher after the ingestion of the protein mix meal, which could have contributed to the decrease in TPR. The reduction in TPR induced by the protein mix was accompanied by increases in HR and CO, which may explain why BP was not significantly affected by the protein mix. Despite these significant haemodynamic changes after the ingestion of the protein mix meal, we did not find significant differences in TPR, CO and HR responses to the ingestion of the protein mix and maltodextrin meals, while we did find a difference in TPR responses in the PROPRES study[7]. It may be that the energy content of the test meals used in the present study was not high enough to detect differences in TPR responses to the ingestion of the test meals. As maltodextrin did not induce a significant change in CO or TPR, it is not clear

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from our data why BP was reduced after the ingestion of the maltodextrin meal.

Our second hypothesis was that higher NO_x responses might be accompanied by increased concentrations of insulin, GLP-1 and glucagon, as these hormones may induce their vasoactive properties via NO-dependent vasodilation[9,10,17]. In accordance with this, the higher NO_x response observed after the ingestion of the protein mix meal was accompanied by higher GLP-1 and glucagon responses. GLP-1 responses observed after the ingestion of dietary proteins have been reported to be either higher than[38] or similar to[39,40] those observed after the ingestion of carbohydrates. However, the study carried out by Li *et al.*[40] measured GLP-1 responses only once after 36 min, while in the present study the difference in GLP-1 responses was detected after 120 min. Karamanlis *et al.*[39] did measure responses at 180 min, but their study may have been underpowered to detect differences in GLP-1 responses, because it included only nine subjects. The higher glucagon response observed after protein consumption was expected, because the maltodextrin-stimulated insulin increase would inhibit glucagon release to maintain glucose homeostasis, while dietary protein is known to be a stimulus for glucagon release[11]. In addition, arginine, which was present in the protein mix, could also have contributed to the higher NO_x response after protein consumption[8]. In our previous study, we had hypothesised that the increase in insulin response after maltodextrin intake could have induced the decrease in BP after maltodextrin intake[7]. In the present study, however, we found no maltodextrin-induced changes in TPR or NO_x responses to support this hypothesis. In addition, no differences were observed in the AIx and ACE activity responses to the ingestion of the maltodextrin and protein mix meals. Therefore, the mechanisms involved in the decrease of BP after maltodextrin consumption remain unknown.

The increased or unchanged BP levels observed after protein consumption in the present study contradict the BP-lowering effect after long-term consumption of dietary proteins that has been reported in many studies[1,2]. However, the PROPRES study has already demonstrated that postprandial responses are not necessarily similar to the effects of long-term consumption[7]. High-carbohydrate diets could increase BP in the long term via insulin-induced Na retention, because insulin reduces hyperglycaemia-induced Na excretion[41]. As we included only participants with fasting glucose levels <7 mmol/l, it is unlikely that this mechanism plays a role in the subjects of the present study. Supporting this, we found no differences in Na excretion in urine samples collected after each test (data not shown). Postprandial responses cannot be directly extrapolated to the effects of chronic protein consumption. Therefore, long-term studies are necessary to determine which protein source could be most beneficial in BP management.

In conclusion, we found no significant differences in TPR responses after the ingestion of the six meals. Higher BP responses were found after the ingestion of the egg-white-protein meal than after that of the pea- and milk-protein meals and lower BP after the ingestion of the maltodextrin meal than after that of the protein mix and sucrose meals. However, larger postprandial reductions in BP after the ingestion of different meals are not necessarily accompanied by higher increases in NO_x concentrations. This is not surprising as BP is regulated by many factors not by NO only. We also found that higher postprandial NO_x responses were not necessarily accompanied by higher insulin, glucagon and GLP-1 concentrations. Mechanisms through which different proteins and carbohydrates acutely affect TPR and BP remain unclear. These data reveal that different protein and carbohydrate sources can induce different postprandial BP-related responses and thus effects of chronic consumption may also differ between different protein sources.

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The authors' contributions are as follows: J. D., M. A. v. B., E. J. B., J. M. G., P. W. d. L. and S. J. L. B. designed the study; J. D., K. F. M. T.-B., and M. A. v. B. conducted the study; K. F. M. T.-B., M. A. v. B. and J. S. analysed the data; K. F. M. T.-B., M. A. v. B., J. D., E. J. B., J. M. G., P. W. d. L., S. J. L. B. and J. S. wrote the article; M. A. v. B. had primary responsibility for the final content. All authors read and approved the final manuscript.

None of the authors has any conflicts of interest to declare.

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