Protein supplementation lowers blood pressure in overweight adults: effect of dietary proteins on blood pressure (PROPRES), a randomized trial

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ABSTRACT
Background: Dietary protein intake may help to manage blood pressure (BP) and prevent complications associated with elevated BP.
Objective: The objective of this study was to determine whether 4 wk of increased protein intake (∼25% compared with ∼15% of energy intake that isoenergetically replaces carbohydrate intake) lowers office and daytime BP.
Design: A randomized, double-blind, parallel study compared consumption of 3 × 20 g protein/d (20% pea, 20% soy, 30% egg, and 30% milk-protein isolate) with 3 × 20 g maltodextrin/d. Protein or maltodextrin were isoenergetically substituted for a sugar-sweetened drink. Primary outcomes were office and daytime BP. A total of 99 men and women [age range: 20–70 y; BMI (in kg/m²): 25–35] with untreated elevated BP (BP ≥130/85 and <160/100 mm Hg) were randomly assigned. Ninety-four completers (51 subjects in the maltodextrin group, 43 subjects in the protein group) were included in the analyses.
Results: Office systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 4.9 ± 1.7 mm Hg (P = 0.005) and 2.7 ± 1.3 mm Hg (P = 0.05) lower in the protein group. Daytime SBP was 4.6 ± 1.7 mm Hg lower in the protein group (P = 0.006), whereas daytime DBP did not differ between groups (P = 0.37). Urinary sodium excretion was higher in the maltodextrin group (P = 0.004).
Conclusion: Increased protein intake, at the expense of maltodextrin, lowers BP in overweight adults with upper-range prehypertension and grade 1 hypertension. This trial was registered at www.trialregister.nl as NTR 1362.

INTRODUCTION
Observational studies indicated that protein intake (1, 2), particularly plant protein intake (3, 4), may be inversely related to BP (5). However, randomized controlled trials on the BP-lowering effects of dietary protein have produced inconsistent results (5–9). In a systematic review, Altorf-van der Kuil et al (10) concluded that increased protein intake may have beneficial effects on BP.

The question of whether it is really the protein fraction of the diet that is responsible for the BP-lowering effect of protein-enriched diets remained because most studies increased the intake of protein-rich foods (6–9) and reduced the intake of carbohydrate-rich foods (6–8) to attain the difference in macronutrient composition. This may have introduced changes in other nutrients or nonnutrients that influenced the BP response. To eliminate these factors as much as possible, we performed the effect of dietary proteins on blood pressure (PROPRES) study, in which an isocaloric difference in protein and carbohydrate intakes was created by providing supplements on top of a standard diet. The protein supplement consisted of a mixture of 4 proteins from different sources, which represented the balance between sources of animal and plant protein in the habitual Dutch diet. We hypothesized that the protein-supplemented diet would lower office and daytime BP over a 4-wk period compared with a maltodextrin-supplemented diet in overweight and obese individuals with untreated upper-range prehypertension or grade 1 hypertension.

SUBJECTS AND METHODS
PROPRES was a 2-arm, double-blind, randomized, parallel trial that compared the BP effects of a diet high in protein to those of a diet high in carbohydrates. The study was conducted in Maastricht (Netherlands) from September 2008 until December 2009. The study was approved by the Medical-Ethical Committee of Maastricht University Medical Center and Maastricht University in Maastricht. All participants gave written informed consent.

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4 Abbreviations used: BP blood pressure; DBP diastolic blood pressure; HR, heart rate; PROPRES, effect of dietary proteins on blood pressure; SBP systolic blood pressure.

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Participants

Participants were overweight [BMI (in kg/m²): 25–35] men and women, aged 20–70 y, with untreated upper-range prehypertension or hypertension grade 1 (SBP of 130–159 mm Hg and/or DBP of 85–99 mm Hg) at the time of random assignment. Participants were nonsmokers with a fasting plasma glucose concentration <7 mmol/L and an estimated glomerular filtration rate (on the basis of plasma creatinine, age, and sex) of ≥60 mL/min⁻¹·(1.73 m²)⁻¹ at screening. Furthermore, participants had stable weight in the 3 mo before the study and did not use vitamin or mineral supplements or take medication that could influence BP. The absence of proteinuria was tested by using a Multistix urine dipstick (Siemens Healthcare Diagnostics BV).

Study protocol

Participant eligibility for the study was established during a screening visit. After inclusion, participants started with a 2-wk run-in period followed by the 4-wk intervention. Each week, participants visited a dietitian who weighed them, measured their BP, advised them on a weight-maintaining diet with a standard composition, and provided them with supplement sachets during the intervention period.

If the BP of participants met the inclusion criteria at the end of the run-in period, participants were stratified for SBP (130–139 and ≥140 mm Hg), sex, and age (<50 and ≥50 y) and randomly assigned to the protein or control group (maltodextrin) with a computer program (MINIM; http://www-users.york.ac.uk/~mb555/guide/minim.htm). Supplement sachets were marked A or B to blind participants and the research staff to the intervention. Supplements were flavored with orange or vanilla flavor to mask the difference in sweetness between test substances. Participants were instructed not to communicate about the taste and texture of supplements with the research staff. At the end of the run-in period and 4-wk intervention, participants collected 24-h urine and measured their ambulatory BP during waking hours (0930–2130) on the same day.

Diet

A dietitian instructed participants to consume a diet that was composed of 15% of energy from protein, 30% of energy from fat, and 55% of energy from carbohydrates and to be moderate with salt intakes. During the 2-wk run-in period, participants were instructed to drink a noncaffeine-containing sugar-sweetened drink (~20 g sucrose) with each meal. Participants were not allowed to consume >2 alcoholic consumptions/d and >4 caffeine-containing consumptions/d.

During the 4-wk intervention period, participants replaced the sugar-sweetened drinks with each meal with 20 g maltodextrin or protein (Agglomix) dissolved in 200 mL H₂O. Thus, 3 × 20 g sucrose was replaced by 3 × 20 g maltodextrin in the control group or 3 × 20 g protein in the protein group. The protein supplement consisted of 20% pea, 20% soy, 30% egg white, and 30% milk-protein isolate. The mixture reflected the ratio between plant and animal proteins (40:60) in the average Dutch diet (11). Protein supplementation increased the protein intake to ~25% of energy and decreased the carbohydrate intake to ~45% of energy. Maltodextrin, which is a glucose polymer, is representative of starch-like foods. Supplements were isocaloric and matched for sodium, potassium, phosphate, magnesium, and calcium contents (Table 1).

Measurements

Primary study outcomes were between-group differences in office and daytime ambulatory BP after the 4-wk intervention. Office BP was measured during the weekly visits. Participants were ≥2 h postprandial at the start of measurements. After 10 min of rest, 3 BP measurements were taken in a sitting position (OMRON 6 comfort; Omron Healthcare Europe BV). The mean of the last 2 measurements was recorded for analysis. The run-in BP was calculated as the average BP measured at the end of the first and second week of the run-in period. The intervention BP was the BP measured at the end of week 4.

Ambulatory BP and HR were measured hourly between 0930 and 2130 at the end of the run-in period and 4-wk intervention. Measurements were done with a validated (12) ambulatory BP monitor (Spacelabs 90207; Spacelabs Healthcare Ltd). The appropriate cuff size was chosen according to arm circumference, and subjects were instructed to hold their arm still during the BP measurement. The LCD display of the BP monitor was inactivated. Participants were instructed to perform their normal daily activities during the measurement day. Prolonged intense physical activity was to be avoided. The BP monitor was set to remove measurements with SBP <70 or ≥260 mm Hg, DBP <40 or ≥150 mm Hg, or pulse pressure <20 or ≥150 mm Hg.

A 24-h urine collection was performed at the end of the run-in and after 4 wk supplementation. An unprocessed urine sample was used for analysis of 24-h urinary sodium, potassium, creatinine, magnesium, albumin, sulfate, and phosphate. Urine was acidified to pH = 2 for analysis of urinary calcium. Unprocessed and acidified urine samples were stored at −80°C until analysis.

Statistical analysis

All analyses were performed with SPSS software (version 19.0; IBM). Values are expressed as means ± SEs. Baseline and

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Supplement composition per serving and intake from supplements per day a</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per serving</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>24.9</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>89</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>20.3</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>0.3</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.7</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>0.2</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>196</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>103</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>148</td>
</tr>
<tr>
<td>Phosphate (mg)</td>
<td>191</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>16</td>
</tr>
</tbody>
</table>

*Supplements were dissolved in 200 mL H₂O and consumed with each meal (3 times/d). During the run-in period, participants consumed 200 mL soft drink or lemonade with each meal (~20 g sucrose).
run-in characteristics were tested with an independent samples t test for between-group differences unless stated otherwise. Weight changes during the intervention were analyzed by repeated-measures ANCOVA with run-in body weight as a covariate. Between-group differences in BP and urinary excretions after 4 wk supplementation were tested with a univariate ANCOVA with the run-in value as the covariate (analysis of completers) or with mixed-model analyses including all participants who were randomly assigned. In the mixed model, run-in BP and BP after 4 wk were analyzed as repeated measures, and an interaction between group and time was tested. Available data from all patients who were randomly assigned were considered in the model, which, thus, included run-in BP from dropouts. The model assumed that BP in dropouts would follow the same course as in completers.

Differences in albumin excretion between groups after the run-in period and after 4 wk supplementation were tested by using Mann-Whitney U tests. Because urine collections are prone to collection errors, changes in urinary excretions were also analyzed after exclusion of subjects with >20% difference in 24-h urinary creatinine excretion between the 24-h urine collected after the run-in period and after 4 wk as an additional covariate. Furthermore, the effect of hypertension status was explored by adding the interaction between hypertension status and supplementation group as a covariate to the initial model. Hypertension status was 1 for upper-range prehypertension and 2 for hypertension. For SBP, participants with SBP ≥140 mm Hg were considered hypertensive, and DBP participants with DBP ≥90 mm Hg were considered hypertensive.

Between-group differences in run-in daytime BP were tested with a linear mixed-model analysis. Differences between groups in daytime (0930–2230) BP and HR after 4 wk were tested with a linear mixed-model analysis, with correction for all run-in daytime BP or HR measurements at the end of the run-in period.

With a sample size of 94 subjects and SD of 7.7 mm Hg in the maltodextrin group and 9 mm Hg in the protein group, the power to show a significant difference (\( \alpha = 0.05 \)) of 5 mm Hg in office SBP between groups was 0.81.

RESULTS

Participants

A total of 123 participants started with the run-in period, of whom 99 subjects were randomly assigned (Figure 1). Five participants in the protein group dropped out during the intervention. Two participants stopped because of immediate adverse effects after consumption of the supplement (one subject experienced nausea, and one subject experienced a lightly swollen face, abdomen, and thighs. Two participants were excluded after randomization because they started with antihypertensive medication, and one subject stopped for personal reasons that were not related to the intervention. A total of 94 participants completed the trial (51 subjects in the maltodextrin group and 43 subjects in the protein group) (Figure 1).

FIGURE 1. Flow diagram of participants in the effect of dietary proteins on blood pressure (PROPRES) study.

Baseline characteristics

The protein and maltodextrin groups were comparable at screening for BMI, age, SBP, DBP, and plasma creatinine. Fasting blood glucose was higher in the maltodextrin group (\( P = 0.05 \)). Run-in office SBP and DBP and daytime SBP and DBP did not differ between groups (Table 2).

Body weight

Repeated-measures analysis showed no significant weight changes (\( P = 0.99 \)) during the intervention. Body weight did not differ between groups during the intervention, with mean values of 85.9 kg in the maltodextrin group and 85.7 kg in the protein group (\( P = 0.35 \)).

Office BP

All between-group differences after 4 wk discussed in the next 3 paragraphs were tested with corrections for run-in values. Office SBP and DBP at run-in and after the intervention are shown in Figure 2. Office SBP was 4.9 ± 1.7 mm Hg (95% CI: 1.5, 8.2 mm Hg) lower in the protein group than in the maltodextrin group (\( P = 0.005 \)) after 4 wk supplementation. DBP was 2.7 ± 1.3 mm Hg (95% CI: 0.1, 5.4 mm Hg) lower (\( P = 0.05 \)) after 4 wk. Analysis of completers included 2 subjects in the protein group who missed their endpoint measurement after 4 wk. BP at the end of week 4 in these subjects was estimated...
from BP changes in the rest of the protein group from weeks 3 to 4. When the last-observation-carried-forward procedure was applied for these 2 subjects, similar results were obtained (not shown). Mixed-model analyses in all 99 subjects who were randomly assigned, which, thus, included the dropouts, resulted in the same conclusions as in the analyses of completers. The effect of supplementation on SBP and DBP was not influenced by hypertension status ($P = 0.47$ and $P = 0.07$, respectively).

**Daytime SBP, DBP, and HR**

After 4 wk supplementation, daytime SBP was $4.6 \pm 1.7$ mm Hg (95% CI: 1.3, 7.9 mm Hg) lower in the protein group than in the maltodextrin group ($P = 0.006$), whereas daytime DBP was comparable in both groups ($P = 0.43$; 95% CI: 3.2, 1.4 mm Hg). The between-group difference in daytime SBP appeared to be as much due to an increase in the maltodextrin group as to a decrease in the protein group (Figure 2). The daytime HR was not different between groups after 4 wk supplementation ($P = 0.52$; data not shown).

**Twenty-four–hour urinary excretions**

Run-in 24-h urine excretions did not differ between groups (Table 3). Urinary markers of protein intake (urea and sulfate excretion) were significantly higher in the protein group after 4 wk supplementation ($P < 0.001$, both). Sodium excretion at 24 h was significantly lower in the protein group than in the maltodextrin group ($P = 0.004$). Potassium excretion at 24 h tended to be lower in the protein group ($P = 0.06$), whereas 24-h calcium excretion tended to be higher in the protein group ($P = 0.07$). Albumin excretion tended to be higher in the maltodextrin group ($P = 0.05$). None of the other urinary excretions differed significantly between groups after 4 wk supplementation (Table 3).

**DISCUSSION**

The PROSPER trial aimed to investigate whether a moderately increased protein intake (+10% of energy) compared with isocalorically increased maltodextrin intake for 4 wk lowers BP in overweight individuals with upper-range prehypertension or grade 1 hypertension. Increased protein consumption was shown to significantly decrease office SBP by 4.9 mm Hg and DBP by 2.7 mm Hg compared with maltodextrin intake. In addition, daytime SBP was 4.6 mm Hg lower in the protein group. If a reduction of 5 mm Hg in office SBP would be attained at the population level, a 14% risk reduction in stroke mortality and
a 9% risk reduction in coronary heart disease mortality are expected (14). Trials that study the effects of BP-lowering antihypertensive agents have also shown risk reductions of 15–22% for major cardiovascular events with modest decreases in BP (15).

The findings in the PROPRES trial are in agreement with those from other large randomized trials that investigated the effects of protein intake on BP (9, 16–18). The effects of an increased protein intake on SBP and DBP in the PROPRES trial were larger than those shown in the OmniHeart trial (15). The effects of increased protein consumption from the effects of reduced carbohydrate consumption. This problem was inevitable because an increased intake of one macronutrient will always result in a decreased intake of at least one of the other macronutrients under isocaloric conditions (10). Supplements were well tolerated by most participants. Two of the 48 subjects in the protein group (4%) experienced adverse effects. One participant felt bloated after consumption of the protein supplement, whereas the other participant experienced nausea. Adverse feelings disappeared when the participants stopped consuming supplements.

### TABLE 3
Twenty-four-hour urinary excretions after run-in period and 4 wk

<table>
<thead>
<tr>
<th>24-h urinary excretion</th>
<th>Run-in</th>
<th>No. of subjects</th>
<th>4 wk</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>M</td>
<td>P</td>
<td>M</td>
</tr>
<tr>
<td>Urea (mmol/d)</td>
<td>442 ± 15$^4$</td>
<td>437 ± 15</td>
<td>38 ± 15</td>
<td>32 ± 15</td>
</tr>
<tr>
<td>Sulfate (mmol/d)</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Potassium (mmol/d)</td>
<td>86 ± 4</td>
<td>83 ± 3</td>
<td>6 ± 2</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>Calcium (mmol/d)</td>
<td>20 ± 0.8</td>
<td>20 ± 0.8</td>
<td>20 ± 0.8</td>
<td>20 ± 0.8</td>
</tr>
<tr>
<td>Sodium (mmol/d)</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
</tr>
<tr>
<td>Magnesium (mmol/d)</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
</tr>
<tr>
<td>Phosphate (mmol/d)</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
</tr>
<tr>
<td>Creatinine (mmol/d)</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
<td>5 ± 0.2</td>
</tr>
<tr>
<td>Albumin (Q1-Q2-Q3; mg/L)</td>
<td>2-4-9</td>
<td>2-4-8</td>
<td>1-4-8</td>
<td>1-4-8</td>
</tr>
</tbody>
</table>

$^1$ P values of between-group differences were calculated by using ANCOVA with run-in as a covariate. M, maltodextrin group; P, protein group; Q, quartile.

$^2$ None of the differences between groups were significant after the run-in period ($P > 0.05$) on the basis of an independent-sample t test (for urea, sulfate, sodium, potassium, calcium, magnesium, phosphate, and creatinine) or Mann-Whitney U test (for albumin).

$^3$ Number of subjects included in the analysis, which was lower than the number of participants because of missing values.

$^4$ Mean ± SE (all such values).

$^5$ P < 0.05.
A strength of this study was that the effects of isolated proteins and carbohydrates, rather than the effects of protein- and carbohydrate-rich foods, were tested, which strengthened the conclusion that it was the increase in protein intake (or reduction in carbohydrate intake) that was responsible for the BP reduction. Because the supplements were matched for micronutrients, confounding effects by these minerals were limited. In addition, body weight was controlled for throughout the whole intervention to avoid a confounding effect of weight changes on BP (26). Both the OmniHeart (9) and Diogenes (27) trials have shown that an increase in protein intake to ~25% of energy is feasible with normal foods. However, it is hard to predict whether such dietary changes that do not involve protein supplementation will lead to comparable results to those shown in the PROPRES study because protein-rich foods also contain other macronutrients and micronutrients, which may affect BP.

In conclusion, the PROPRES study shows that BP can be lowered by increasing the intake of proteins in exchange for carbohydrates in the context of an isocaloric weight-maintaining diet for 4 wk in overweight subjects with upper-range prehypertension and grade 1 hypertension.

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