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Ingestion of Broccoli Sprouts Does Not Improve Endothelial Function in Humans with Hypertension

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Abstract

Ingestion of glucosinolates has previously been reported to improve endothelial function in spontaneously hypertensive rats, possibly because of an increase in NO availability in the endothelium due to a attenuation of oxidative stress; in our study we tried to see if this also would be the case in humans suffering from essential hypertension.

Methods: 40 hypertensive individuals without diabetes and with normal levels of cholesterol were examined. The participants were randomized either to ingest 10 g dried broccoli sprouts, a natural donor of glucosinolates with high in vitro antioxidative potential, for a 4 week period or to continue their ordinary diet and act as controls. Blood pressure, endothelial function measured by flow mediated dilation (FMD) and blood samples were obtained from the participants every other week and the content of glucosinolates was measured before and after the study. Measurements were blinded to treatment allocation.

Results: In the interventional group overall FMD increased from 4% to 5.8% in the interventional group whereas in the control group FMD was stable (4% at baseline and 3.9% at the end of the study). The change in FMD in the interventional group was mainly due to a marked change in FMD in two participants while the other participants did not have marked changes in FMD. The observed differences were not statistically significant. Likewise significant changes in blood pressure or blood samples were not detected between or within groups. Diastolic blood pressure stayed essentially unchanged in both groups, while the systolic blood pressure showed a small non significant decrease (9 mm Hg) in the interventional group from a value of 153 mm Hg at start.

Conclusion: Daily ingestion of 10 g dried broccoli sprouts does not improve endothelial function in the presence of hypertension in humans.

Trial Registration: Clinicaltrials.gov NCT00252018

Introduction

Oxidative stress is considered to play a important role in the processes of cellular injury which ultimately leads to the development of atherosclerosis.[1] However, clinical studies of antioxidants directly administered as a supplement have shown no effects of antioxidants on cardiovascular morbidity and mortality. [2]. An attractive alternative could be to augment the naturally occurring antioxidative defense.[3,4]

A group of substances which might have this effect are the glucosinolates, substances naturally occurring in cruciferous plants; the glucosinolates are precursors to isothiocyanates, of which sulphoraphane is one of the most potent inducers of the expression of phase-2-enzymes, in mammalian cells.[5,6,7]. Among the enzymes induced in vitro the glutathione-S-reductase may be of particular interest since it is instrumental in recycling glutathione to its reduced state in the cell. In broccoli sprouts younger than 4 days glucoraphanin[5] is abundant and when ingested, glucoraphanin is converted to sulphoraphane. In vitro studies have demonstrated the beneficial effects of glucosinolates on mammalian cell lines[7]. Wu and colleagues demonstrated that daily treatment of Stroke Prone Hypertensive rats with dried broccoli sprouts after weaning attenuated the development of hypertension and improved endothelial function of the carotid arteries.[8]
We therefore decided to study whether the administration of dried broccoli sprouts in patients with essential hypertension could decrease blood pressure and increase flow mediated vasodilation (FMD). We have focused on endothelial function since this parameter previously has been demonstrated to be influenced by the level of oxidative stress, and because it is a sensitive predictor for oxidative stress as well as for the future development of atherosclerosis.[9,10]

We hypothesized that an increase in flow mediated vasodilation should be detectable in the interventional group after four weeks of treatment both if we used the participants as their own controls and if we compared them to a control group. This was our primary outcome.

Secondary outcomes in our study were measurement of blood pressure and lipoproteins where we were interested in seeing if the blood pressure would decrease during treatment and if lipoproteins would show an increase in HDL as well as a decrease in LDL.

Methods

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1.

Participants

Volunteers were enrolled in the study after advertising in local newspapers for non smoking hypertensive individuals without known diabetes or hypercholesterolemia. In- and Exclusion criteria are listed in Table 1.

Ethics

The study was approved of the ethics committee for Copenhagen and Frederiksberg municipalities (no 01-257/04). Written informed consent was obtained from all participants.

Intervention

Endothelial function was measured on each individual three times, at start of treatment and twice during the four weeks of treatment with an interval of two weeks. Blood samples were obtained for analyzing the blood content of erythrocyte content blood glucose and inflammatory status was to insure that the stimulus for measuring FMD was as uniform as possible for each visit since a change in blood viscosity could influence the shear stress of the vessel wall and therefore increase the measured FMD, likewise elevated blood glucose has been showed to decrease FMD response. Endothelial function was measured using Flow Mediated Dilation (FMD) using a protocol following current guidelines.[11,12] The participants were instructed to arrive for the investigation after fasting for the last 12 hours including medication. After arrival they were placed in supine position for at least 10 minutes before initiation of measurements. The examination room was quiet and had a temperature of about 22°C and had subdued lighting. Measurements were performed on the brachial artery of the right upper arm approximately 10 cm above the antecubital fossae with the pneumatic tourniquet placed distally to the ultrasound transducer immediately below the antecubital fossae at the area of greatest width of the forearm. The tourniquet was inflated to a pressure of 300 mm Hg for a period of 4 minutes and 45 seconds. During the last 15 seconds of inflation and the first 15 seconds after deflation of the tourniquet, a Doppler spectroscopy of the flow through the brachial artery was recorded, permitting calculation of the flow increase through the brachial artery. As a control, the flow independent dilation (FID) was measured 10 minutes after the measurement of FMD by administering 100 μg nitroglycerin sublingually. Measurement of FMD and FID were given as the relative increase in diameter in percent compared to the baseline diameter of the brachial artery. In order to assess the possible changes of shear stress during the study, blood samples were analyzed for the relative content of red blood cells and inflammatory markers (leukocytes and c-reactive protein) since these factors usually are the most important contributors to the viscosity of the blood.[13] The measurements were performed on an Acuson XP10/4 ultrasound apparatus using a 7 MHz linear transducer (L7), and the images were analyzed real time on a personal computer using a NI PCI-1470 frame grabber and Vascular Image Acquisition (VIA) software.[14,15] The visits were made on the same time of the day in order to avoid diurnal variation of FMD.

Procurement of sprouts

The broccoli sprouts were obtained from a commercial grower (Van Der Plass Sprouts BV, the Netherlands) and were sprouted for 3 days. Immediately after harvesting they were refrigerated.
and subsequently dried at 40°C. The dried sprouts were afterwards packaged in airtight plastic bags each containing 10 grams of dried sprouts, equivalent to 100 g fresh sprouts. The dried sprouts had the glucosinolate content measured using previously described methods[16,17]. In accordance with the study by Wu et al. [8] it was attempted to construct a placebo for the control group by freezing/thawing sprouts before drying. However this preparation still contained large amounts of glucosinolate. Therefore the study was conducted without placebo treatment of the control group, which served as time-control. Before the start of the study and after the analysis of the glucosinolate content we did consider if it would be better to use another type of sprouts for the control group in order to keep participants as well as investigators blinded. We decided again against this because we were not able to provide such a placebo with taste and smell as the broccoli sprouts, and because we were not able to get a placebo which would be identical to the broccoli sprouts in all aspects but the glucosinolate content.

Randomization and design

Patients were randomised to treatment with broccoli sprouts or control in a 1:1 ratio. Treatment consisted in a daily ingestion of 10 g dried broccoli sprouts. The block size for randomization was 4. Randomization was performed by an assistant in sealed envelopes and group allocation was blinded to the investigator. The participants were thus unblinded with regards to their allocation but the investigator was blinded throughout the study to the status of the participants. Since broccoli sprouts are not commercially available and have a much larger content of glucoraphanin than mature broccoli, we were confident that the control group would not ingest glucoraphanin in substantial amounts during the study. Pre-study antihypertensive medications were maintained during the study for all participants. The participants were given a package containing 28 bags with 10 g dried broccoli sprouts in each, instructions were to eat the content with one of the meals of the day, they were also instructed to avoid eating or drinking for at least 8 hours prior to each day of study. As part of our efforts to determine compliance we took blood samples from the participants at each session and the participants were informed that this also was done in order to estimate the content of the active substances in their blood stream. The investigator was blinded during the study and the preliminary statistical analysis, During the study the analysis of blood samples and measurement of FMD was done immediately after each session and the data were entered into the study database. The investigator was unaware of the allocation of the participants during the study. After conclusion of the study the allocation of each participant into two groups was given the investigator, but during this part it was still not revealed which group had been the control and which had been the interventional group.

Objectives and Outcomes

Following the hypothesis outlined in the introduction our primary outcome was if we would observe an increase in FMD in the interventional group during the intervention. Secondary outcomes were changes in blood pressure and changes in levels of blood cholesterol during the study in the interventional group.

Statistical Methods

The results were analyzed using SAS software (version 9.10). Comparisons between intervention and control group were analysed by non-paired Student’s t-test and comparison of changes over time for the intervention and the control group were performed using a paired students test. Results were presented with calculated mean values and confidence intervals for the measurements at the different session as well as for the differences between values in the two groups.

| Table 2. Baseline characteristics of the control and interventional group. |
|---------------------------------|----------|-----------|----------|----------|----------|----------------|
| Male                            | Control  | Sd         | Range    | Intervention | SD        | Range   | p       |
| Male                            | 5        | n/a        | n/a      | 11         | n/a       | n/a     | 0.10    |
| Female                          | 15       | n/a        | n/a      | 10         | n/a       | n/a     | 0.50    |
| Age                             | 54       | 10         | 32–67    | 58         | 9         | 38–77   | 0.23    |
| Cigarettes(Packet years)        | 6.9      | 17         | 0–70     | 6          | 10.4      | 0–41    | 0.84    |
| BMI                             | 26.2     | 3.2        | 22–33.7  | 29.1       | 6.6       | 21–51   | 0.08    |
| Systolic mean                   | 158.6    | 20.4       | 132–215  | 158.5      | 20.9      | 121–196 | 0.98    |
| Diastolic mean                  | 98       | 7.8        | 88–115   | 96         | 10.4      | 80–115  | 0.50    |
| Plasma Glucose                  | 5.3      | 0.4        | 4.5–5.9  | 5.4        | 0.4       | 4.5–6.4 | 0.40    |
| total Cholesterol               | 5        | 0.7        | 3.6–7.1  | 5.1        | 1         | 3.6–7.6 | 0.55    |
| HDL                             | 1.7      | 0.5        | 0.9–2.8  | 1.7        | 0.6       | 1.1–3.4 | 0.85    |
| LDL                             | 2.9      | 1          | 2–4.2    | 3          | 0.8       | 1.7–5.1 | 0.32    |
| Triglycerides                   | 1.09     | 0.7        | 0.43–3.59| 1.03       | 0.6       | 0.38–2.5| 0.71    |
| Antihypertensive drugs          | users    | users      | p        | users     | users    | p        |
| C03 Diuretics                   | 4        | n/a        | n/a      | 5         | n/a       | n/a     | 0.77    |
| C07 β-Blockers                  | 2        | n/a        | n/a      | 2         | n/a       | n/a     | 0.96    |
| C08 Calciumantagonists          | 7        | n/a        | n/a      | 3         | n/a       | n/a     | 0.12    |
| C09 ACE inhibitors or ATII antagonists | 8 | n/a          | n/a       | 12        | n/a       | n/a     | 0.27    |

Since all participants were non smokers at the time of the investigation, packet-years refers to years of smoking prior to the study. Antihypertensive drugs are grouped by ATC.

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It was assumed that a detectable effect would produce at least a 3% difference in FMD, on this assumption it was calculated that our study would have a power of 90% with 20 individuals in each group.

**Results**

**Participant flow**

The study enrolled 41 participants, 20 in the control group and 21 in the interventional group. In all 53 persons were screened prior to enrollment, of these 10 did not meet the enrollment criteria and 2 decided not to participate. The reason for including one more in the interventional group was that one participant dropped out one day after receiving the sprouts owing to the disagreeable taste of the sprouts. One participant in the interventional group missed one visit because of hospitalization (leg fracture). All other participants received treatment as allocated and participated in examinations as planned. The baseline characteristics for the participants are listed in Table 2 and the participant flow diagram is presented in Fig. 1.

**Vascular measurements**

The FMD values with 95% confidence interval and p values calculated using students test for the interventional and control group are displayed in Table 3; overall, no significant changes occurred in the two groups during the period of treatment. The change in mean FMD between weeks 0 and 4 is greater by 2.25% in the intervention group compared with the control group. The 95% confidence

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**Table 3. Development of FMD in percent during the study.**

| Week | 0   | 2   | 4   | Change 0–4 |
|------|-----|-----|-----|-----------|
|      | Intervention Mean | 4.07 | 4.12 | 5.80    | 1.78     |
| CI   | 2.75 to 5.39     | 2.93 to 5.31 | 3.19 to 8.41 | -0.95 to 4.41 |
| Control Mean | 4.08 | 3.60 | 3.56 | -0.52     |
| CI   | 2.70 to 5.46     | 2.55 to 4.64 | 2.74 to 4.38 | -1.99 to 0.94 |
| Difference Mean | -0.01 | 0.53 | 2.24 | 2.25     |
| CI   | -1.88 to 1.84    | -1.01 to 2.06 | -0.46 to 4.98 | -0.73 to 5.23 |

Mean and confidence interval (2.5–97.5%) none of the differences were significant on the 0.05 level using Students Test.

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interval for this difference between changes is (−0.73%; 5.23%).

FMD was slightly lower in the control group as seen by Fig. 2, and
the average vascular diameter was slightly lower in the control
group (data not shown). Figure 2 shows boxplots of the FMD values
in the interventional as well as the control group, of notice is that the
median values for the FMD in either group did not change during
the study. In order to visualize the causes for the observed changes
in the interventional group for week 0 to 4, we have in Figure 3
plotted a histogram for the distribution of changes in the FMD
values in the interventional group between week 0 and week 4.

Blood Samples
For the blood test taken simultaneously with the FMD
measurements, we have not been able to detect any differences in
these samples in the course of our study. The results for are shown in
Table 2 and 4 We present the values at at randomization, as
baseline values. The variation we observe in these blood samples
appeared basically to be of a random nature and we were not able to
demonstrate any systematic change during the experiment.

Development in blood pressure
Systolic, diastolic as well as mean blood pressure did not change
significantly during the study. Differences detected in the study
were related to gender and age.
Results are shown in Tables 5 and 6.

Glucosinolate content of the broccoli sprouts
The glucosinolate content of the sprouts was determined before and
after the drying and packaging had taken place in order to ensure that
the content of glucosinolates in the dried sprouts was known and to
assess the possible deterioration of the glucosinolates due to the drying.
Measurements of the concentration in the sprouts at the end of
the study were similar to those prior to the study (Table 7). The
sprouts had a glucoraphanin content of 25.9±8.5 μmol/g dry
weight and a total glucosinolate content 48.5±14.2 μmol/g dry weight.

As stated in the methods section blood samples were drawn at each visit in order to determine the glucosinolate content in the blood of the participants. Analysis of these samples did not show detectable levels of glucosinolates or isothiocyanates in any of the participants.

### Adverse events

Apart from one participant leaving the study because of the taste of the sprouts which made her very uncomfortable, no adverse effects were observed during the study.

### Discussion

In the current study we have tested whether dietary glucosinolates can improve endothelial function in patients with hypertension as it has been demonstrated in animal models. Using supplements of broccoli sprouts with preserved concentrations of the glucosinolate glucoraphanin, we found no significant change in endothelial function, measured twice during an investigator-blinded 4-week treatment period. We are aware of the fact that a change does take place in the interventional group between week 0 and week 4 even if this change is insignificant. An issue could therefore be if this lack of significance was due to insufficient power of the study. We find this unlikely, since, as demonstrated in Figure 3 it is very few of the participants in the interventional group who actually increase their peak dilation during the study. At Figure 3 it can also be seen, that the majority of the observed changes cluster around 0 with 75% having a change in FMD of less than 3%, our stated minimum observable difference. If we look at the distribution of the absolute difference in peak diameter for week 0 and 4 we find a similar pattern with 75% having a peak diameter difference of 0.26 mm or less, values which are very close to the measuring uncertainty of the method. As such we find it justified to state, that we do not observe an increase in FMD in our study group.

During this period, we have not been able to demonstrate any change in blood pressure, or blood samples reflecting blood lipids or inflammatory status. This finding is at odds with experiments carried out on spontaneously hypertensive stroke prone rats.

### Table 4. Mean, confidence intervals and p-values for selected blood samples, p-values calculated using Students test.

| Total Cholesterol | Intervention | Control |
|-------------------|--------------|---------|
| Weeks             | mean Cl 2.5  | CI 97.5 | mean Cl 2.5  | CI 97.5 | p-value |
| 0                 | 5.08 4.62    | 5.53 4.95 | 4.65 5.24 | 0.62 |
| 2                 | 5.07 4.57    | 5.57 4.88 | 4.62 5.14 | 0.49 |
| 4                 | 5.04 4.55    | 5.53 4.50 | 4.76 5.36 | 0.94 |

| HDL               | Weeks       | mean Cl 2.5  | CI 97.5 | mean Cl 2.5  | CI 97.5 | p-value |
|-------------------|-------------|--------------|---------|--------------|---------|---------|
| 0                 | 1.73 1.49   | 1.97 1.81   | 1.54 2.07 | 0.67 |
| 2                 | 1.67 1.40   | 1.94 1.73   | 1.50 1.96 | 0.72 |
| 4                 | 1.65 1.40   | 1.90 1.78   | 1.54 2.01 | 0.45 |

| LDL               | Weeks       | mean Cl 2.5  | CI 97.5 | mean Cl 2.5  | CI 97.5 | p-value |
|-------------------|-------------|--------------|---------|--------------|---------|---------|
| 0                 | 2.76 2.29   | 3.22 2.78   | 2.51 3.04 | 0.94 |
| 2                 | 2.72 2.26   | 3.19 2.72   | 2.48 2.95 | 0.98 |
| 4                 | 2.89 2.49   | 3.28 2.80   | 2.53 3.06 | 0.70 |

| Haemato-chrите     | Week        | mean Cl 2.5  | CI 97.5 | mean Cl 2.5  | CI 97.5 | p-value |
|-------------------|-------------|--------------|---------|--------------|---------|---------|
| 0                 | 0.39 0.34   | 0.43 0.58   | 0.20 0.96 | 0.31 |
| 2                 | 0.41 0.39   | 0.42 0.40   | 0.39 0.41 | 0.17 |
| 4                 | 0.40 0.39   | 0.42 0.39   | 0.38 0.40 | 0.24 |

Mean and confidence interval (2.5–97.5%) none of the differences were significant on the 0.05 level using Students Test.

### Table 6. Development of Diastolic Blood Pressure during the study.

|          | 0  | 2  | 4  | Change 0–4 |
|----------|----|----|----|------------|
| Intervention | Mean | 89.70 | 90.58 | 88.65 | −1.05 |
| CI        | 83.73 to 95.66 | 84.88 to 96.28 | 84.04 to 93.26 | −8.18 to 6.08 |
| Control   | Mean | 91.80 | 89.55 | 88.75 | −3.05 |
| CI        | 86.53 to 97.07 | 85.32 to 93.78 | 84.84 to 92.66 | −9.12 to 3.02 |
| Difference| Mean | −2.10 | 1.03 | −0.10 | 2.00 |
| CI        | −9.80 to −5.80 to −5.95 to −7.06 to 5.60 | 7.91 | 5.75 | 11.06 |

Mean and confidence interval (2.5–97.5%) none of the differences were significant on the 0.05 level using Students Test.

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(SHSP), which may be due to a variety of factors. The animal study was designed to investigate if a daily dose of sprouts would protect against the development of hypertension, while we only studied endothelial function in patients with established hypertension. Furthermore, dosage as well as duration of treatment was also different, as the rats were given dried sprouts from weaning and until termination of the experiment after fourteen weeks, which translated to a human lifespan would imply treatment form early childhood into adolescence. Finally, hypertension is not a well described disease entity in humans whereas the SHSP are a distinct breed of rats characterized by their tendency to develop hypertension and stroke.[18,19] It is therefore very likely that the distinct breed of rats characterized by their tendency to develop hypertension and stroke.

Table 7. Concentration of glucosinolates in the sprouts prior to and after the study.

| Glucoraphanin | Prior to study (Mean μmol/g) | D | proportion | Mean (μmol/g) | SD | proportion |
|---------------|-----------------------------|---|------------|---------------|---|------------|
| 30.3          | 4.0                         | 75.0% | 25.9      | 8.5           | 53.4% |
| Total         | 40.4                        | 5.8  | 100.0%    | 48.5          | 14.2 | 100.0% |

All concentrations are given as μmol/g with the proportion signifying how large a proportion of the glucosinolate content consisted of Glucoraphanin. doi:10.1371/journal.pone.0012461.t007

Supporting Information

Checklist S1 CONSORT Checklist.

Found at: doi:10.1371/journal.pone.0012461.s001 (0.19 MB DOC)

Protocol S1 Trial Protocol, in Danish.

Found at: doi:10.1371/journal.pone.0012461.s002 (0.10 MB DOC)

Author Contributions

Conceived and designed the experiments: BBC BK CRM TSH LK CTP HD. Performed the experiments: BBC NBM BK. Analyzed the data: BBC

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