Glycocalyx, cardiometabolic disease and inflammation

Broekhuizen, L.N.

Citation for published version (APA):
Reduction of MCP-1 and MIF by a Polyphenol-rich extract in subjects with clustered cardiometabolic risk factors

British Journal of Nutrition, in press

LN Broekhuizen (1)
DF van Wijk (1)
H Vink (2)
A Stalmach (3)
A Crozier (3)
BA Hutten (1)
JJP Kastelein (1)
PG Hugenholtz (4)
W Koenig (5)
ESG Stroes (1)

(1) Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands
(2) Department of Physiology, University of Maastricht, Maastricht, the Netherlands
(3) Centre for Population and Health Sciences, University of Glasgow, Glasgow, United Kingdom
(4) Department of Cardiology, Erasmus University, Rotterdam, the Netherlands
(5) Department of Internal Medicine – Cardiology, University of Ulm Medical Center, Ulm, Germany
Abstract

Inflammatory activation contributes to the progression of the metabolic syndrome as well as atherogenesis. Hence, anti-inflammatory strategies are of potential interest. Since NF-κB activation plays a key role in regulating the immune response and polyphenols (PF) have been shown to inhibit NF-κB transcription in vitro, we set out to evaluate the impact of polyphenol rich extract on the chronic inflammatory state as well as the acute immune responses in subjects with clustered metabolic risk factors.

The effect of 500 mg daily polyphenol rich extract per os (Frutologic, also known as VinitroxTM) or placebo for 4 weeks was tested in a randomized, placebo-controlled, double-blind cross-over study in 34 subjects with 2 or more metabolic risk factors. During the final study visit, the effect of an inflammatory challenge (LPS 1 ng/kg bolus) on the inflammatory response was evaluated in a random subgroup of subjects (N=24).

Four weeks daily polyphenol rich extract treatment had a modest impact on inflammatory cytokines with a reduction of MCP-1 by 6.5 % (PF 116 pg/ml [97-136] vs. placebo 124 pg/ml [105-153]; p<0.05, median, [interquartile range]) and of MIF by 10.8 % (PF 2512 pg/ml [1898-3972] vs. placebo 2814.5 [2296-3852]; p<0.05). No differences were found between the polyphenol rich extract and placebo group for other chemo- and cytokines. We observed a 48% reduction of MCP-1 production over 6 hours (PF 766 ± 155 ng/ml vs. placebo 1466 ± 989 ng/ml; p<0.05, area under the curve (AUC)), in the polyphenol rich extract treated group (N=12) compared to the placebo group (N=12) in patients after an in vivo LPS challenge.

In conclusion, short-term polyphenol rich extract administration had a modest anti-inflammatory effect in subjects with clustered metabolic risk factors. Further dose-ranging studies are required in order to evaluate whether and what dose of polyphenols may be beneficial in patients with cardiometabolic diseases.
**Reduction of MCP-1 and MIF by a Polyphenol-rich extract in subjects with clustered cardiometabolic risk factors**

**Introduction**

Inflammation plays a critical role not only in the development of atherosclerosis, but also in the pathogenesis of atherosclerotic complications such as myocardial infarction and stroke. In line, elevated levels of pro-inflammatory chemo- and cytokines have been associated with increased cardiovascular risk. Therefore, anti-inflammatory strategies may hold a promise for cardiovascular prevention strategies on top of current lipid-lowering interventions. The metabolic syndrome, a major risk factor for cardiovascular disease, is also characterized by a chronic low-grade inflammatory state. The latter has been attributed to inappropriate adipocyte enlargement accompanied by increased stress in the endoplasmatic reticulum of the adipocytes, eventually leading to activation of NF-κB and subsequent production of down-stream pro-inflammatory cytokines and chemokines such as interleukin 6 (IL-6), tumour necrosis factor α (TNF-α), macrophage migration inhibitory factor (MIF) and monocyte chemoattractant protein (MCP)-1. These pro-inflammatory cytokines are thought to modulate the paracrine function of adipocytes, further contributing to systemic inflammation and insulin resistance in patients with obesity.

**Polyphenols**

Polyphenols (PF) are chemical substances found in plants containing two or more phenol groups. In vitro, PF have been shown to inhibit TLR-mediated inflammatory responses via the MyD88 independent TLR3 and TLR4 signalling pathways, diminish NF-κB activation and reduce the production of downstream chemo- and cytokines. In addition, anti-oxidative and anti-thrombotic effects have been reported. Consistent with the anti-atherogenic effects in vitro, PF administration attenuates atherosclerotic lesion formation in a variety of animal studies. In humans, however, the results of PF are heterogeneous. For instance, one study reported a promising effect of PF on the carotid intima-media thickness of atherosclerotic patients. However, positive consequences of PF ingestion for cardiovascular disease still need confirmation from clinical trials evaluating hard endpoints. Furthermore, most evidence comes from epidemiological studies and there are very few randomized placebo controlled trials that have investigated effects of PF consumption in patients with an increased risk for cardiovascular diseases (CVD). In the present randomized, placebo-controlled, double-blind study, we evaluated the effect of oral intake of 500 mg of polyphenol rich extract (Frutologic/Vinitrox®, Bio Serae) daily during 4 weeks on circulating inflammatory mediators in 34 men and women with clustered cardiovascular risk factors. In addition, we challenged a subgroup with a low dose of endotoxins to monitor the effect of PF on the immune response to an acute inflammatory stimulus.
Methods

Participants
The 34 participants of the study were recruited from the outpatient clinic of the Academic Medical Center (Amsterdam, the Netherlands) and through poster advertisement. The subgroup for the LPS challenge consisted of 24 participants that were randomly chosen and asked for consent at the first visit of the study. Men and women were eligible to participate if 2 or more of the following criteria were present: a waist circumference of $\geq 102$ cm for men or $\geq 88$ cm for women, triglyceride levels $\geq 1.69$ mmol/L, HDL-C $\leq 1.03$ mmol/L for men or $\leq 1.29$ mmol/L for women, a systolic blood pressure $\geq 130$ mmHg, a diastolic blood pressure $\geq 85$ mmHg and a glucose level $\geq 6.1$ mmol/L, according to the ATP III criteria [Adult Treatment Panel III, attributed to NCEP/NHLBI]. During the study period, participants were instructed to refrain from wine and grape containing beverages, large amounts of tea, fruit juice, and dark chocolate. The use of lipid-lowering medication or antioxidants was not allowed throughout the study. Subjects were excluded if they reported coronary heart disease (CHD), stroke, malignancies, or chronic inflammatory diseases in their medical history. Subjects who were current smokers or who were known with alcohol abuse were excluded from the study.

Design
The study was designed as a double-blind, placebo controlled, randomized cross-over trial. During the screening visit, two weeks prior to the first study visit, blood was withdrawn for measurement of baseline characteristics. Participants were randomized to daily 500 mg polyphenol rich extract obtained from grapes and apples (Frutologic, also known as Vinitrox™, BioSerae) for 4 weeks or placebo. The polyphenol rich extract was for 100% composed of a mixture of grape and apple mass. High performance liquid chromatography revealed 400 ppm of epsilon-vineferin, and a total polyphenols content of 112.7 % / dry mass (catechin) was detected by a 280 nm UV spectrophotometer. Important polyphenols present in grapes and apples are anthocyanins, proanthocyanidins, resveratrol, flavanol monomers and flavonoids, which are mainly present in skin and seeds of purple coloured grapes. Following a 4 week washout period, participants were switched to placebo or polyphenol rich extract, respectively. The daily amount of polyphenol rich extract and placebo was dosed in capsules of 250mg. Participants were prescribed to take two capsules a day in the morning, just after breakfast. At the end of each treatment period, blood was drawn for laboratory testing; e.g. total cholesterol, LDL, HDL, glucose, HbA1C, CRP, Haemoglobin and Creatinine. Blood pressure was measured and endothelial function (flow mediated dilatation [FMD] by ultrasound) was assessed [see below]. All measurements were performed after an overnight fast. At the end of the second treatment period, a subgroup of participants was challenged with a low dose of endotoxin from Escherichia coli of 1 ng/kg of bodyweight of endotoxin (Escherichia coli lipopolysaccharide [O113:H10], 10.00EU/ vial, PDS #67801, Lot number 67466 (production date 4.1.97), Cape Cod incorporated/ National Institutes of Health, Bethesda, Maryland, United States) as published previously. Blood for laboratory analysis
was withdrawn from the contralateral antecubital vein at 1, 3, 4, 6 and 8 hours after the infusion. Vital signs were measured at regular intervals; every 15 minutes in the first 3 hours, incidence, time, and severity of symptoms were recorded by the study physician. FMD measurements were performed at baseline and 4 hours after LPS challenge. Approval for this study was obtained from the internal review board of the Academic Medical Center, Amsterdam, the Netherlands. The study was carried out in accordance with the principles of the Helsinki Declaration. All participants gave written informed consent.

**Measurements**

**Laboratory**

Baseline serum concentrations of total cholesterol, HDL cholesterol and triglycerides were measured in fresh serum samples by standard enzymatic methods (Roche Diagnostics, Basel, Switzerland). LDL cholesterol concentrations were calculated using the Friedewald formula. Glucose was assessed using the hexokinase method (Gluco-quant, Hitachi 917, Hitachi). HbA1C was measured by HPLC (Reagens Bio-Rad Laboratories, Veenendaal, the Netherlands) on a Variant II (Bio-Rad Laboratories). Plasma aliquots were snap-frozen and stored at -80°C for shipment to the Biomarker Laboratory at the University of Ulm, Germany. Plasma C-reactive protein (CRP) levels were measured with a high sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany). Tumor necrosis factor-α (TNF-α), Interleukin-6 (IL6), Adiponectin, Oxidized LDL, Monocyte chemoattractant protein-1 (MCP-1) and macrophage Migration Inhibitory Factor (MIF) levels were measured using a commercially available sandwich ELISA kit (R&D Systems, Abingdon, UK). The intra and inter-assay coefficients of variation of quality control test sera were <10% and <20% respectively.

**FMD**

Each patient underwent measurement of flow mediated dilation of the left brachial artery by B-mode ultrasound imaging using an Acuson Aspen (Siemens, Mountain View USA) ultrasound system with a L7, 5-10 MHz linear array broadband transducer (centre frequency 7.0MHz). The FMD of all 34 participants was measured after each treatment period. The FMD of all 24 participants in the subgroup was measured again 4 hours after the LPS infusion. FMDs that did not pass our quality protocol were excluded from the analysis by a blinded reviewer. Patients were instructed to refrain from food, alcohol and any other drink except water prior to the measurements. Subjects were scanned in the supine position, with the left arm stabilized in an integrated arm holder. A blood pressure cuff was placed on the left forearm from the medial epicondyle downwards. The scan of the left brachial artery started with 1 minute of continuous baseline recording, followed by 5 minutes of forearm ischemia, induced by inflating a vascular pressure cuff to 250 mmHg. Hyperemic blood flow was induced by deflating the cuff, after which 3 minutes of continuous ultrasound recording followed. Images were acquired with ECG triggering on every R-wave to capture the frames in the diastolic phase of the brachial artery. Semi-automated qualitative and quantitative image analysis of scans was performed off-line by an
experienced image analyst using dedicated software (Brachial Analyzer, MIA vascular tools, Coralville USA). Images were blinded for treatment and visit. The average baseline diameter and the maximum post-cuff deflation diameter were used to calculate the percentage flow-mediated vasodilation (%FMD). %FMD was defined as: %FMD = 100 (maximum post cuff deflation diameter / baseline diameter).

**Statistical analysis:** Data are presented as arithmetic mean ± standard deviation when normally distributed and as median with corresponding interquartile range for values with a skewed distribution. A paired samples t-test was used for normally distributed baseline values, whereas a Wilcoxon rank test for paired measurements was employed to test for skewed baseline variables. For the cross-over study we used a repeated measures mixed model with time and treatment as fixed effects. An interaction term of time and treatment was used to test for possible carry-over effects. For the LPS intervention we calculated the production of circulating mediators after LPS by the area under the curve (AUC) from the time of the infusion (t=0) until 6 hours after the start of the challenge (t=6). An unpaired samples t-test was used for normally distributed values, whereas a Mann-Whitney U test was employed to test for skewed variables. Analyses were performed with SPSS version 16.0 (Chicago, IL, USA). A p-value < 0.05 was defined as statistically significant.

**Results**

**Baseline characteristics of the participants**

Baseline characteristics of the study participants are listed in Table 1. A total of 34 participants were included; 21 men and 13 women. Of all participants, 44% had two, whereas 56% had 3 ATP-III (Adult Treatment Panel III, attributed to NCEP/NHLBI) defined risk factors of the metabolic syndrome. The prevalence of risk factors was distributed as follows: increased waist circumference 88%, hypertriglyceridemia 50%, low HDL-C levels 27%, elevated blood pressure 88% and elevated plasma glucose levels 29%. The average age was 58.3 ± 7.9 years. Baseline characteristics of the participants were: BMI 31.9 ± 4.8 [kg/m²], systolic blood pressure 147 ± 18.3 [mmHg], diastolic blood pressure 89 ± 9.3 [mmHg], total cholesterol 5.6 ± 1.1 [mmol/L], LDL-C 3.3 ± 0.9 [mmol/L], HDL-C 1.3 ± 0.4 [mmol/L], triglycerides 1.76 [1.38-3.23] [mmol/L], glucose 5.6 [5.1-6.1] [mmol/L], HbA1c 5.8 ± 0.5 [%] and CRP 2.1 [1.1-2.8] [mg/L]. During the second visit, 24 (15 men and 9 women) of the total 34 participants were challenged with a low dose LPS.

**Effect of polyphenols on inflammatory parameters**

Following 4 weeks of polyphenols administration, BMI and blood pressure were comparable between the polyphenol rich extract and placebo group whereas LDL cholesterol levels were slightly higher in the polyphenol rich extract treatment group (Table 1). No significant interaction term was found indicating
that no carry-over effects were present in our study. We found a statistically significant reduction of 6.5% for MCP-1 and of 10.8% for MIF levels in the polyphenol rich extract treated group versus the placebo treated group. In contrast, no changes were observed for other markers of metabolic disease and inflammation (e.g. IL6) between the polyphenol rich extract treatment period and the placebo treatment period of all participants (Table 1). Also, FMD was comparable in both groups which is consistent with the latter findings (Figure 1).

Table 1. Markers for cardiovascular risk and inflammation according to treatment period of all 34 participants.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Polyphenols</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>58.3 ± 7.9</td>
<td>58.3 ± 7.9</td>
<td>ns</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.9 ± 4.9</td>
<td>31.6 ± 5.2</td>
<td>ns</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>142.5 ± 16.8</td>
<td>143.0 ± 14.7</td>
<td>ns</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>86.1 ± 10.3</td>
<td>87.0 ± 8.9</td>
<td>ns</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.57 ± 1.29</td>
<td>5.71 ± 1.19</td>
<td>ns</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.39 ± 1.18</td>
<td>3.56 ± 1.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.32 ± 0.31</td>
<td>1.32 ± 0.33</td>
<td>ns</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.26 [1.33-2.39]</td>
<td>1.88 [1.21-2.41]</td>
<td>ns</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>6.0 [5.6-6.8]</td>
<td>6.1 [5.7-6.9]</td>
<td>ns</td>
</tr>
<tr>
<td>HbA1C, %</td>
<td>5.8 ± 0.4</td>
<td>5.8 ± 0.4</td>
<td>ns</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>2.37 [1.36-3.94]</td>
<td>2.43 [1.19-5.03]</td>
<td>ns</td>
</tr>
<tr>
<td>Oxidized LDL, U/ml</td>
<td>91.0 [77-118]</td>
<td>91.5 [79-116]</td>
<td>ns</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>1.55 [1.21-1.79]</td>
<td>1.38 [1.1-1.83]</td>
<td>ns</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>124 [105-153]</td>
<td>116 [97-135.8]</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MIF, pg/ml</td>
<td>2814.5 [2296-3852.3]</td>
<td>2511.5 [1898-3972]</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Adiponectin, µg/ml</td>
<td>8.0 [6.3-10.8]</td>
<td>8.5 [5.3-11.8]</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or median [interquartile range] of 34 patients. BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, HbA1C = glycated hemoglobin, CRP = C-reactive protein, IL6 = interleukin 6, MCP-1 = monocyte chemoattractant protein 1, MIF = macrophage migration inhibitory factor.

Effects of PF-rich extract on LPS response

Twenty-four participants received an inflammatory challenge of LPS of 1 ng/kg bodyweight during the final visit. All participants showed a modest rise in temperature with concomitant side-effects including chills, accelerated heart rate, and headache. These symptoms did not differ between the polyphenol rich extract and the placebo group. The ensuing peak increases in other inflammatory parameters were not significantly different [not displayed]. However, total MCP-1 production was significantly reduced in subjects of the polyphenol rich extract treatment group. In agreement with previous publications, FMD was reduced 4 hours after LPS administration [Figure 1] in both groups. However, no differences in FMD were observed between the polyphenol rich extract and placebo treated subjects.
Figure 1A. Area under the curves [AUCs] of monocyte chemoattractant protein 1 (MCP-1). Production of MCP-1 after an inflammatory challenge is significantly lower in polyphenol abstract rich treated patients as compared to treatment with placebo; 766.1 ng/ml vs. 1465.5 ng/ml (p=0.04).

Figure 1B. Flow mediated dilatation [FMD] of the left brachial artery was not significantly different between the placebo and the polyphenol rich abstract group; 4.28 ± 0.49 and 4.69 ± 0.52 (p=ns) respectively, n=25. FMD did show a significant difference before and 4 hours after the LPS infusion in the subgroup; 5.56 ± 0.7 and 3.84 ± 0.43 (p<0.05), n=14. Data are presented as mean ± SE. [* = p≤0.05]
Discussion

In the present study, we show that 4-weeks administration of 500 mg polyphenol rich extract daily was associated with a significant, albeit modest reduction in circulating MCP-1 and MIF levels in subjects with clustered metabolic risk factors without affecting other inflammatory markers. The inflammatory response following low-dose LPS challenge lowered MCP-1 production [AUC] over 6 hours in the polyphenol rich extract treated individuals, whereas no differences could be detected for other inflammatory cytokines. Although, the present findings do support a modest anti-inflammatory effect of polyphenol rich extract in subjects with clustered metabolic risk factors, the clinical relevance of these findings needs further validation in larger studies with prolonged follow-up.

Polyphenols and baseline inflammatory markers

Polyphenol rich extract from various food sources, including alcoholic beverages made from grapes, have been shown to have a variety of beneficial effects, including anti-oxidative, anti-thrombotic and vasodilatory effects. In addition, polyphenol rich extract have been reported to reduce the inflammatory response particularly for toll-like receptor (TLR)-3 and -4 specific ligands. Since the metabolic syndrome is characterized by a low-grade inflammatory state, in which a high fat diet related postprandial increase of endotoxins may play a role, we focused on patients with metabolic risk factors in the present study to assess a potential anti-inflammatory effect of polyphenols in vivo. Following 4 weeks of supplementation with a polyphenol rich extract, a significant, albeit modest reduction in circulating levels of MCP-1 and MIF was observed. Our present findings are in correspondence with previous reports corroborating the anti-inflammatory capacity of polyphenol rich extract in animal models. Similarly, the phenolic compound resveratrol was shown to inhibit NF-κB transcription in vitro. In healthy volunteers, consumption of polyphenol rich extract in alcoholic beverages has also been reported to reduce NF-kB activation as well as MCP-1 plasma levels during a high fat diet. Both MCP-1 and MIF are considered important pro-inflammatory cytokines associated with increased cardiovascular risk. MCP-1 is an important chemokine, attracting monocytes to sites of endothelial injury, and both MCP-1 serum levels as well as MCP-1 genotype are described to be associated with cardiovascular disease. Mice receiving pharmacologic blockade of MCP-1 and mice deficient in MCP-1 develop less atherosclerosis compared to placebo treated or wild-type mice. MIF has been associated with atherogenesis as well as risk factors associated with the development of metabolic disorders such as obesity and insulin resistance. MIF is a pleiotrope cytokine that acts, amongst others, as a pro-inflammatory cytokine which is released as a consequence of the systemic stress response. Both MIF and MCP-1 play a crucial role in the chemotaxis of monocytes into the adipose tissue, which is a key step in the progression towards ‘dysfunctional’ adipose tissue, characterized by a disregulation of adipocyte paracrine function. Finally, we did not find a difference in other markers associated with a proatherogenic state between the polyphenol rich extract and the placebo treated groups. Based on the present findings, one might speculate whether polyphenol rich
extract may positively affect ‘dysfunctional’ fat tissue in patients with metabolic syndrome after long-term treatment10.

Polyphenol and inflammatory reaction following LPS challenge
In addition to the effect of polyphenol rich extract on the inflammatory state in patients with clustered metabolic risk factors, we measured the effect of polyphenol rich extract on inflammatory markers and cytokines after provocation with a low dose of LPS. Consistent with the effects of polyphenol rich extract on the chronic inflammatory state, low dose LPS challenge resulted in a significant lower MCP-1 area under the curve (AUC) over 6 hours in the polyphenol rich extract treated group compared to the placebo treated group. Again, we did not find an effect on other inflammatory markers. Monagas et al. recently described that pre-treatment of peripheral blood mononuclear cells with certain phenolic metabolites induced a reduction of TNF-α, IL-6 and IL-1β production after LPS stimulation by more than 80% 53. Another in vitro study found differential effects between the various phenolic compounds that where used. For instance, a reduction of IL1β could be detected for addition of oleuropein glycoside, present in olive oil, but not for other PF used. No effect was seen on TNF-α or IL-6 54, the latter finding being more similar to the results of the present study. It has been suggested that not all PF have anti-inflammatory characteristics and that different PF have specific anti-inflammatory properties, which could in part explain the heterogeneous results in earlier studies 55.

Limitations
The results of the present study warrant several comments. First, a treatment period of 4 weeks is a relatively short period and the dose of 500 mg per day is probably suboptimal as bioavailability of many polyphenolic substances is poor 56. Second, frutologic is a polyphenol rich mixture of compounds which includes various substances and may even enclose potential non-polyphenolic compounds. This was a first exploratory study to investigate the effect of polyphenol rich extract on acute inflammation in obese participants with multiple cardiovascular risk factors. However, despite the short treatment and the small number of 34 included participants, we were able to detect a significant effect on both basal MCP-1 and MIF plasma levels, and the acute MCP-1 response after a low dose endotoxin challenge.

Conclusions
The present study shows that oral ingestion of Frutologic, a polyphenol mixture derived from apples and grapes, can decrease circulating pro-inflammatory cytokines, both involved in the pathogenesis of adipocyte dysfunction and atherogenesis. Considering the important role for the infiltration of monocytes into fat tissue and the subsequent inflammatory response, PF might have beneficial effects for the development and/or progression of cardiovascular disease in patients with clustered metabolic risk factors. However, this needs further confirmation in larger trials investigating the optimal dose and the long-term results of PF for the development and progression of metabolic risk factors and CVD.
Reference List


Reduction of MCP-1 and MIF by a Polyphenol-rich extract in subjects with clustered cardiometabolic risk factors


Addendum

Glycocalyx dimension in patients with clustered risk factors for cardiometabolic disease

LN Broekhuizen [1]
ESG Stroes [1]
H Vink [2]

[1] Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands
[2] Department of Physiology, Maastricht University, the Netherlands
In addition to the anti-inflammatory effects of the PF-rich extract Frutologic/Vinitrox®, we evaluated whether ingestion of PF-rich extract would also have an effect on the endothelial glycocalyx. Glycocalyx dimension was assessed in subjects with clustered cardiovascular risk factors after administration of PF-rich abstract versus placebo. Both groups were compared to healthy controls. Sidestream darkfield imaging (SDF/OPS) was used to estimate the mean diameter of the red blood cell column (RBC) as well as to calculate the perfused boundary region (PBR) of the RBC (figure 1). Theoretically, an increase in PBR, i.e. the RBC permeable part of the glycocalyx, indicates a perturbation of healthy glycocalyx function (Chapter 4).

**Figure 1.** PBR = perfused boundary region, RBC = red blood cell column, Dperf = the red blood cell perfused diameter and PBR.
Glycocalyx dimension was measured in 21 randomly selected participants of the PRIME study after every treatment period: 4 weeks PF-rich extract and 4 weeks placebo. The results of both study periods were compared to a standard control group of 21 healthy individuals who were not included in the PRIME study. We hypothesized that glycocalyx function would be decreased in participants with multiple risk factors for cardiovascular disease (CVD) and that 4 weeks of treatment with PF-rich extract would lead to an improvement in glycocalyx dimension.

Results

Glycocalyx dimension in 21 healthy subjects with normal BMI, blood pressure, glucose and cholesterol levels was compared to 21 participants of the PRIME study after treatment with PF-rich extract and after placebo. Mean age of healthy controls was: 61 ± 9.4 compared to 57.8 ± 10.6 (PRIME participants). Median RBC radius in controls was used as a standard for comparison and set at 0.00 µm [-0.50 µm (p10) – 0.32 µm (p90)].

Glycocalyx dimension at baseline
Median PBR in controls was 0.09 µm [-0.41 µm – 0.72 µm]. Median PBR in PRIME participants after placebo as well as after treatment with PF-rich extract was significantly larger compared to healthy controls: 0.92 µm [-0.21 – 1.52] p=0.002 (placebo) and 0.56 µm [-0.04 µm – 1.07 µm] p=0.003 (PF-rich extract) (Figure 2). RBC radius was smaller in the placebo group compared to controls: -0.38 µm [-0.94 µm – 0.09] vs. 0.00 [-0.50 µm - 0.32 µm] p=0.01.

Figure 2. Median PBR was larger in PRIME participants after placebo (p=0.002) and after treatment with PF-rich extract (p=0.003) compared to controls.
Glycocalyx dimension following PF-rich extract
PBR after treatment with PF-rich extract showed no decrease compared to placebo: 0.56 µm [-0.04 µm – 1.07 µm] (PF-rich extract) vs. 0.92 µm [-0.21 µm – 1.52 µm] (placebo) p = ns (Figure 2). In contrast, RBC radius after PF-rich extract increased significantly compared to placebo: -0.13 µm [-0.63 µm - 0.32 µm] (PF-rich extract) vs. -0.38 µm [-0.94 µm – 0.09 µm] (placebo) p=0.04. RBC radius normalised towards the level of controls after administration with PF-rich extract -0.13 µm [-0.63 µm - 0.32 µm] vs. 0.00 µm [-0.50 µm - 0.32 µm] p=ns (Figure 3).

Figure 3. RBC radius was smaller in the placebo group compared to controls (p=0.01). Median RBC-radius normalised towards the level of controls after PF-rich extract administration (p=ns).

Discussion
The present data shows that the PBR of red blood cells was increased in participants with multiple risk factors for CVD compared to healthy controls, pointing towards a perturbation of the endothelial glycocalyx. Interestingly, RBC was decreased in participants with multiple risk factors for CVD after placebo. Following 4 weeks of PF-rich extract administration, RBC radius increased significantly, whereas the PBR remained unaffected.

Glycocalyx changes in subjects with cardiometabolic risk factors
The increase in PBR in participants with multiple risk factor for CVD confirms previous studies showing loss of glycocalyx dimension in conditions associated with increased risk for CVD such as type II diabetes, chronic low-grade inflammation and dyslipidemia. These conditions induce endothelial dysfunction, ultimately giving rise to accelerated atherogenesis. Malfunction of the endothelial glycocalyx layer is probably one of the earliest alterations in this cascade. The present study shows that this process has already started in subjects with an increased body weight and one or more risk factors for cardiometabolic disease according to the ATP III criteria.
Addendum to Chapter 7; the PRIME study

**Glycocalyx changes after PF-rich extract**

PRIME participants displayed an increased PBR, both after PF-rich extract administration and placebo, compared to healthy controls. The latter indicates that the RBC is able to penetrate deeper into the glycocalyx layer in subjects with multiple risk factors for CVD, compatible with a loss of glycocalyx dimension and a smaller barrier between circulating blood cells and the endothelium. Administration of PF-rich extract for 4 weeks was, however, not able to restore this loss of glycocalyx dimension, despite the beneficial changes measured in circulating markers of inflammation (Chapter 7).

In contrast, median RBC increased significantly after administration of PF-rich extract compared to placebo. It has previously been described in rats that loss of glycocalyx dimension after an infusion with the glycocalyx degradating enzyme hyaluronidase coincides with increased pericapillary edema. Pressure of increased extravascular fluid is known to decrease the diameter of capillaries, giving rise to a smaller diameter of the observed RBC. An increase in RBC diameter with a concomitantly unaltered PBR following PF-rich extract suggests a reduction in the permeability of the glycocalyx with ensuing reduced pericapillary edema rather than a change in glycocalyx dimension, as attested to by the unchanged PBR. Further studies are needed to validate this effect of PF-rich extract on glycocalyx permeability.

**Conclusion**

Participants of the PRIME study had larger PBR’s then controls suggesting a perturbed endothelial glycocalyx enabling blood cells to penetrate closer to the vascular wall. The RBC in PRIME subjects after placebo was significantly smaller compared to controls, whereas RBC diameter increased following PF-rich extract administration. It is tempting to speculate that this is due to less pericapillary edema in the PF-rich extract treated group, indicating a decrease in vascular permeability following PF-rich extract administration.

**Acknowledgements**

We gratefully acknowledge Carmen Vlahu, department of Nefrology AMC, for providing glycocalyx data of 21 healthy controls.
Reference List


