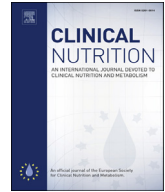




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## Randomized Control Trials

## The effect of monomeric and oligomeric FLAVAnols in patients with type 2 diabetes and microalbuminuria (FLAVA-trial): A double-blind randomized controlled trial



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## SUMMARY

**Background & aims:** Microalbuminuria is an early sign of vascular complications of type 2 diabetes and predicts cardiovascular disease and mortality. Monomeric and oligomeric flavanols (MOFs) are linked to improved vascular health. The aim of this study was to assess the effect of 3 months MOFs on albuminuria and endothelial function markers in patients with type 2 diabetes and microalbuminuria.

**Methods:** We conducted a double-blind, placebo-controlled trial among patients with type 2 diabetes and microalbuminuria. Patients with type 2 diabetes received either 200 mg MOFs or placebo daily on top of their habitual diet and medication. The primary endpoint was the between-group difference of the change in 24-h Albumin Excretion Rate (AER) over three months. Secondary endpoints were the between-group differences of the change in plasma levels of different markers of endothelial dysfunction. Mixed-modelling was applied for the longitudinal analyses.

**Results:** Participants (n = 97) were 63.0 ± 9.5 years old; diabetes-duration was 15.7 ± 8.5 years. Median baseline AER was 60 (IQR 20–120) mg/24 h. There was no within-group difference in median change of AER from baseline to 3 months in the intervention (0 (-35-21) mg/24 h, p = 0.41) or the control group (0 (-20-10) mg/24 h, p = 0.91). There was no between-group difference in the course of AER over three months (log-transformed data: β = -0.02 (95%CI -0.23-0.20), p = 0.88), nor in the plasma levels of the endothelial dysfunction markers.

**Conclusion:** Daily 200 mg MOFs for three months on top of habitual diet and usual care did not reduce AER and plasma markers of endothelial dysfunction compared to placebo, in patients with long-term type 2 diabetes and microalbuminuria.

**Clinical trials registration:** NTR4669, [www.trialregister.nl](http://www.trialregister.nl).

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**Abbreviations:** AER, Albumin excretion rate; ARB, Angiotensin receptor blocker; CVD, Cardiovascular disease; eGFR, Estimated glomerular filtration rate; MOFs, Monomeric and oligomeric flavanols; RAS-I, renin-angiotensin system inhibitors; RCT, Randomized controlled trial; sICAM-1, Soluble intercellular cell adhesion molecule-1; sVCAM-1, Soluble vascular cell adhesion molecule-1; T2D, Type 2 diabetes; vWF, Von Willebrand Factor.

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## 1. Introduction

Type 2 diabetes (T2D) and the concomitant micro- and macrovascular complications are among the health care challenges of the 21st century [1]. Microalbuminuria is an early sign of these complications. In 20–40% of these cases, the condition progresses to diabetes-related nephropathy within ten years [2–4]. Diabetes-related nephropathy has become the leading cause of end-stage renal disease [5]. Microalbuminuria is associated with vascular dysfunction, cardiovascular disease (CVD), and mortality in individuals with and without T2D [6,7]. In a recent post-hoc analysis of the LEADER trial, a >30% reduction in albuminuria was associated with fewer cardiovascular events and less nephropathy [8]. The authors described decreasing albuminuria as a great unused potential [8]. The mechanism behind the link between microalbuminuria and CVD is not known. Microalbuminuria may reflect a generalized endothelial dysfunction of the microvasculature, with increased transvascular protein leakage and a pro-atherogenic state [9].

Endothelial dysfunction increases the release of soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular cell adhesion molecule 1 (sICAM-1), and von Willebrand Factor (vWF). These circulating, endothelium-derived proteins are biomarkers of vascular complications and are validated predictors of the progression of nephropathy in T2D [10].

Medication aimed at microvascular protection in T2D include fibrates [11], SGLT2-inhibitors [12], GLP-1 receptor agonists [12] and antihypertensives [13]. Lowering blood pressure, preferably with inhibitors of the renin-angiotensin system (RAS-I), reduces microalbuminuria. However, remission of albuminuria by treatment with RAS-I is generally incomplete, and side effects, renal and liver insufficiency may limit RAS-I use. This warrants the search for complementary therapeutic or dietary approaches to treat or alleviate microalbuminuria. In this regard, extracts containing monomeric and oligomeric flavanols (MOFs) offer a dietary management opportunity to diminish albuminuria in patients with long-term T2D.

The human diet contains a broad variety of monomeric, oligomeric, and polymeric forms of flavanols [14]. The MOFs are relatively abundant in products like red wine, cocoa, tea, legumes, and the bark, peels, seeds, and skins of many plants [15]. In general, the average intake of various flavonoids (the family to which flavanols belong), in the Western diet is below the dietary amounts that appear to have health effects [16]. In this regard, MOFs' beneficial effect on reversing endothelial dysfunction and maintaining cardiovascular health have been widely addressed in the past decades [17–24]. In epidemiological studies, the dietary flavanol intake has been linked to reduced T2D and CVD risk [17,18]. In intervention studies, grape seed extract products, containing various amounts of MOFs, improved cardiovascular health markers in healthy volunteers and patients with risk factors for CVD, like metabolic syndrome, hyperlipidemia, and hypertension [19–21]. In patients with T2D, beneficial effects were observed on chronic kidney disease, non-proliferative diabetes-related retinopathy, markers of oxidative stress, inflammation, and glycemia [22–25]. The mechanisms of action through which MOFs lead to improved endothelial function imply protecting the vascular-wall collagen and elastin fibers against damaging triggers that cause vascular hyperpermeability, like oxidative stress and inflammation [26]. Also, MOFs have direct antioxidant and free radical scavenging activities and inhibit lipid peroxidation [27]. We hypothesized that patients with T2D might benefit from grape-seed derived MOFs by improving vascular function and thereby slowing down or mitigating the micro- and macrovascular complications present in patients with long-term T2D. Therefore, we performed a randomized controlled trial to

determine the effect on renal function and markers of endothelial function of a daily intake of 200 mg MOFs during three months, on top of usual care in patients with T2D and microalbuminuria.

## 2. Materials & methods

### 2.1. Study design

The FLAVA-trial was a double-blind, randomized controlled trial. The protocol of this trial has been published previously [28]. Adult patients with T2D and microalbuminuria were recruited between December 2014 and April 2019 from the outpatient diabetes clinics of four health care centers in Rotterdam, the Netherlands. These included one tertiary (Erasmus University Medical Center), two secondary (Havenziekenhuis and Ikazia Ziekenhuis), and one primary health care clinic (general practice 'Gezond op Zuid'). The inclusion criteria were: T2D, age between 40 and 85 years, and microalbuminuria in the previous 6 months or longer. Microalbuminuria was defined as excretion of 30–300 mg albumin in a 24-h (24 h) urine sample or as 3.5–35 mg for females and 2.5–25 mg for males albumin/mmol creatinine in a random urine portion [29]. Exclusion-criteria were: diabetes mellitus other than type 2; consumption of any specific dietary product that provides daily MOFs in an amount of  $\geq 25$  mg/day in the month prior to inclusion; use of anti-coagulation medicines; major health conditions like an organ transplant, untreated cancer, current chemotherapy or radiotherapy, acute or chronic organ failure; microalbuminuria due to other conditions than T2D; pregnancy or lactation during the trial. All participants signed informed consent. Patients would not interrupt or change their regular dietary pattern and regular medication.

Eligible participants were randomized to either the intervention or the control group, in an allocation ratio of 1:1. The block randomization was computer-controlled and supervised by a statistician who was not involved otherwise in the trial. All investigators, medical staff, statisticians, and participants were blinded to the allocation.

This study has been reviewed and approved by the Medical Ethics Committee of Erasmus Medical Center Rotterdam (reference number MEC-2014-426/NL49572.078.14). The study was conducted according to the principles of the Declaration of Helsinki (version October 2013, Brazil) and in accordance with the Dutch Medical Research Involving Human Subjects Act (WMO). All participants provided written informed consent.

### 2.2. Intervention

Participants in the intervention group received daily a dry extract from grape seeds (*Vitis vinifera*) containing 200 mg MOFs (Masquelier's® OPCs, INC Agency BV, Loosdrecht, The Netherlands) for a period of three months. The product contains 200 mg of catechins and epi-catechins (single flavan-3-ols) and oligomeric flavan-3-ol units (dimers to pentamers) per sachet. The manufacturer's quality control process guarantees the quality and standardized amount of the MOFs-fractions. The dose of MOFs and the duration of the intervention were based on data from previous prospective human studies performed with the same MOFs compound, showing favorable effects on vascular health factors in healthy male smokers at 200 mg daily over a 2 month period [30,31]. Participants were instructed to consume the product dissolved in water daily for three months. The control group received a daily placebo, which was identical in package and color to the research product but did not contain MOFs. The exact formulation of the investigational product and placebo can be found in [Supplementary Table 1](#). Participants received one box containing

the sachets needed for the three-month study period. Study visits were scheduled at baseline, at 6 weeks and at 3 months of intervention. Each participant was contacted by telephone between the first and the second study visit to monitor possible problems and to encourage proper use of the product.

During the trial, all participants received usual care provided by their diabetes team and had to continue their prescribed medications, allowing for non-MOF-related adjustments when required. Since this may potentially affect our outcome measurements, all used medications, dosage modifications, and other medical interventions taking place during the trial were recorded. We asked the participants to keep their lifestyles unchanged during the trial. Other than the usual standard T2D-related dietary restrictions, there were no dietary restrictions during the study, except for the exclusion of dietary-products containing  $\geq 25$  mg MOFs other than the research product.

### 2.3. Outcome measures

The primary endpoint was the change over time in Albumin Excretion Rate (AER) during three months of intervention compared to placebo. Twenty-four-hour urine samples for AER measurements were collected at baseline, at six weeks and three months. The albumin level in the 24 h-urine samples was measured by immunoturbidimetric assay (Roche Diagnostics GmbH, Mannheim, Germany). The secondary endpoints were the between-group differences in the change over time from baseline to three months of plasma sVCAM-1, sICAM-1 and vWF. Blood samples were collected in EDTA at baseline and three months. Plasma was isolated and stored at  $-80^{\circ}\text{C}$  until analysis. Plasma levels of sVCAM-1, sICAM-1, and vWF were determined by a Human Magnetic Luminescence Assay (R&D Systems Inc., Minneapolis, USA). The other parameters (urine creatinine, serum creatinine, glycated hemoglobin (HbA1c), fasting glucose, and lipids) were determined by standard clinical chemistry assays. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation formula [32]. Anthropometric parameters were measured at baseline and three months.

Information on demographic variables and lifestyle was collected using standard questionnaires. Food frequency questionnaires were applied to estimate the amounts of dietary MOFs consumption. The amount of the consumed MOFs was determined using The Phenol-Explorer database version 3.0 (<http://phenol-explorer.eu/>) [33]. “Composite vascular complications” was defined as: having any micro- or macrovascular complication other than microalbuminuria (since every participant in the trial had microalbuminuria). To monitor adverse effects and determine compliance, self-developed questionnaires were used at six weeks and three months.

### 2.4. Statistical analysis

All analyses were conducted according to the intention-to-treat principle. Continuous data are expressed as the mean  $\pm$  SD if normally distributed or as median (25–75th percentiles) if non-normally distributed. Categorical data are expressed as percentages. Differences in categorical data between the groups were analyzed by chi-square test. In case of continuous data and when two independent groups, the unpaired t-test (normally distributed) or Mann-Whitney-U (non-normally distributed) were used, and when two dependent observations, the Wilcoxon-Signed-Rank test was used. In case of continuous data in more than two groups, the Friedman-test was used.

Mixed-modelling was applied for the longitudinal analyses of logarithmically transformed AER, sVCAM-1, sICAM-1, and vWF values. There were two levels in the models: the participants

constitute the upper level, their repeated measures the lower level. For each outcome variable, a model was postulated, using treatment group, time, and treatment–time interactions as fixed effects. In addition, we adjusted the models for age, sex and BMI. The deviance statistic [34] using restricted maximum likelihood [35] was applied to determine whether the covariance structure should include slope and intercept–slope interaction next to the intercept. A p-value  $<0.05$  was considered significant. The analyses were carried out using IBM SPSS statistics version 25.

The sample size was calculated based on the following: considering a clinically relevant effect of 20% change of AER, we estimated an average AER of 100 decreasing to 80 mg/24 h ( $\pm$ SD 40) in the intervention group, compared to no expected change in the control group. With a two-sided alpha set at 0.05, power at 0.80, and a correlation of 0.70 between the three repeated measurements [36], we calculated that at least 48 patients in each study group, i.e. 96 in total, were required.

## 3. Results

### 3.1. Baseline characteristics

In this study, 98 patients with T2D and microalbuminuria were enrolled. Figure 1 shows the flow chart of the study. One patient was excluded from the analysis after randomization, because of acute, progressive, diabetes-related complications and subsequent major changes in the anti-diabetes and anti-hypertensive medication shortly after inclusion. No serious adverse events of the intervention product or the placebo have been reported.

Baseline characteristics are shown in Table 1. A total of 36 (37.1%) participants were enrolled from Erasmus University Medical Center, 28 (28.9%) from Havenziekenhuis, 22 (22.7%) from Ikazia Ziekenhuis, and 11 (11.3%) from Gezond op Zuid. The age was  $63.0 \pm 9.5$  (range 40–84) years, 37 (38.1%) were female. The duration of diabetes since diagnosis was  $15.7 \pm 8.5$  (range 0–40) years and 73.7% of the patients depended on insulin injections. Eighty percent of the participants indicated to use lipid lowering medication, predominantly statins. Plasma LDL and TG levels were well above the targets recommended by the ESC and EASD [37]. Fifty (52.1%) of the participants had one or more vascular complications on top of microalbuminuria. The median baseline AER was 60 (IQR 20–120) mg/24 h. The median baseline MOFs consumption through habitual diet was 77.4 (IQR 43.5–223) mg/day. RAS-I was used by 69 participants (73.4%), of which ACE-inhibitors were used by 31 participants. There were no significant differences in the baseline characteristics including medication between the two groups (Table 1).

### 3.2. Within-group changes in outcomes during the intervention

In the intervention group, the median change in AER from baseline to 3 months was 0 (–35–21) mg/24 h ( $p = 0.41$ ). In the control group, the median change in AER was 0 (–20–10) mg/24 h ( $p = 0.91$ , Table 2). Similarly, the within-group changes in the median plasma levels of sVCAM-1, sICAM-1 and vWF were not significant for both groups (Table 2).

### 3.3. Between-group differences in outcomes during the intervention

The between-group difference in the evolution of AER over the three months of the intervention was not significant (log-transformed outcome:  $\beta = -0.02$  (95%CI –0.23–0.20),  $p = 0.88$ ). Adjustment for sex, age and BMI did not change these results (Table 3 and Supplementary Tables 2 and 3). Additional adjustments for the baseline use of MOFs and RAS-I medication did not change these results (data not shown).

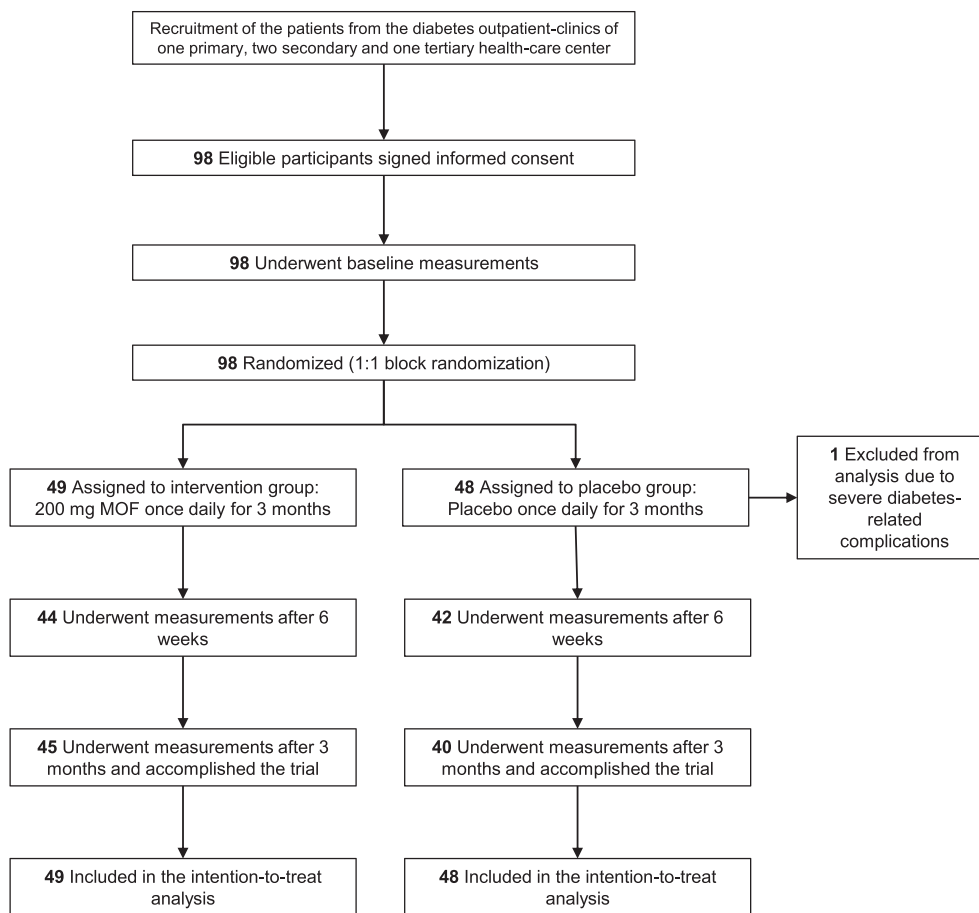


Fig. 1. Flow-chart of the trial.

The between-group difference in plasma sICAM-1, sVCAM-1, and vWF levels over the three months of intervention was not significant (log-transformed outcome:  $\beta = -0.00$  (95%CI -0.10-0.09),  $p = 0.95$ ;  $\beta = -0.15$  (95%CI -0.39-0.09),  $p = 0.21$  and  $\beta = 0.14$  (95%CI -0.25-0.52),  $p = 0.48$ , respectively). Adjustment for sex, age, and BMI did not change these results (Table 3).

Post-hoc analyses were performed to assess the interaction effects of the changes in the study parameters over time with site of enrolment, and with the number of vascular complications. No significant interaction effects were found between the change of AER, sICAM-1, sVCAM-1, and vWF over time and enrolment-site ( $p = 0.43$ ,  $p = 0.07$ ,  $p = 0.92$ , and  $p = 0.81$ , respectively). Also, no significant interactions were found between the change of AER, sICAM-1, and sVCAM-1 over time and composite vascular complications ( $p = 0.15$ ,  $p = 0.74$ , and  $p = 0.31$ , respectively). However, there was an interaction between changes in plasma vWF levels and vascular complications ( $p = 0.016$ ), which indicated a decreased vWF level in the control group in patients with more vascular complications. Subgroup analysis of the change in AER showed highly similar results in participants younger versus older than the median age of 65.5 years in the intervention arm as well as in the control arm (all  $p > 0.05$ ).

#### 4. Discussion

In this double-blind, randomized controlled trial (RCT), we did not find an effect on AER and endothelial function-markers after three months of daily 200 mg MOFs derived from grape seeds on

top of habitual diet and medication in patients with T2D and microalbuminuria.

Our findings are in line with another RCT showing no beneficial effect, although on different outcomes, of supplementation with grape seed extract in patients with T2D [38]. In the latter study, a two-month intake of 200 mg grape seed extract daily did not affect total antioxidant capacity, superoxide dismutase, glutathione peroxidase in red blood cells, and malondialdehyde levels [38]. In contrast, three other RCTs showed beneficial effects of grape seed extract supplementation in patients with T2D [22–24]. In an RCT with 33 participants with diabetes-related chronic kidney disease and hypertension, 23 patients received 2100 mg grape seed extract daily for six months. They had borderline significantly improved glomerular filtration rate, less albuminuria, and a better antioxidant status compared to 10 patients on placebo [22]. This study was the only other study in patients with complicated T2D. Turki et al. [22] analyzed many biomarkers; and correction for three tests already removes the findings. Notably, the authors described the grape seed extract dosage as low and expected that ten times higher dosages would be required to obtain more significant effects. In another RCT with 86 participants, 150 mg grape seed extract daily for 12 months, significantly improved non-proliferative diabetes-related retinopathy [23]. In the third RCT with 32 participants, 600 mg grape seed extract daily for four weeks showed significant improvements in oxidative stress and inflammation, and glycemia in patients with T2D [24]. The differences in results between these studies and our study may be due to 1) difference in dosage and specific active compounds, 2) the difference in study endpoints, 3) duration of the intervention, and 4)

**Table 1**  
Baseline characteristics of the participants.

Characteristic <sup>a</sup>	All participants (n = 97)	Intervention (n = 49)	Control (n = 48)
<b>Age, years</b>	63.0 ± 9.5	62.5 ± 10.1	63.6 ± 8.9
<b>Sex, female, n (%)</b>	37 (38.1)	20 (40.8)	17 (35.4)
<b>Duration type 2 diabetes, years</b>	15.7 ± 8.5	15.6 ± 8.6	15.7 ± 8.5
<b>Weight, kg</b>	91 ± 20.5	88.7 ± 20.7	93.3 ± 20.3
<b>BMI kg/m<sup>2</sup></b>	30.6 ± 6.0	30.1 ± 6.0	31.2 ± 6.1
<b>Waist/Hip ratio</b>	0.99 ± 0.1	0.98 ± 0.08	1.0 ± 0.1
<b>SBP, mmHg</b>	140.7 ± 18.6	139 ± 19.3	136 ± 15.2
<b>DBP, mmHg</b>	77 ± 10.8	76.4 ± 12	77.6 ± 9.4
<b>MAP, mmHg</b>	98.2 ± 11.4	97.2 ± 12.7	99.2 ± 9.9
<b>Plasma creatinine, umol/l</b>	85.2 ± 22.8	82.4 ± 20.5	88.3 ± 25.1
<b>eGFR, ml/min/1.73 m<sup>2</sup></b>	78.1 ± 18.3	80.3 ± 18.6	75.6 ± 18
<b>TG, mmol/l</b>	2.6 ± 2.2	2.8 ± 2.3	2.4 ± 2.0
<b>Total-C, mmol/l</b>	4.2 ± 1.0	4.4 ± 1.3	3.9 ± 0.6
<b>HDL-C, mmol/l</b>	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3
<b>LDL-C, mmol/l</b>	2.4 ± 1.0	2.5 ± 1.0	2.3 ± 0.9
<b>HbA1c, %</b>	8.2 ± 3.4	8.3 ± 3.5	8.1 ± 3.2
<b>HbA1c, mmol/mol</b>	66.3 ± 13.4	67.3 ± 14.8	65.2 ± 12
<b>sICAM-1, ng/ml</b>	64.3 (40-96.6)	64.9(43.8-113.6)	59.5 (35.8-90.4)
<b>sVCAM-1, ng/ml</b>	704 (544-886)	726 (564-869)	664 (530-895)
<b>vWF, IU/ml</b>	391 (283-476)	384 (264-449)	393 (286-492)
<b>AER, mg/24 h</b>	60 (20-120)	80 (27-120)	60 (18-120)
<b>Alb/creat, mg/mmol</b>	6.1 (2.2-12.7)	6.4 (2.5-12.6)	5.4 (2.2-12.9)
<b>Anti-hypertensive use</b>			
RAS-I (%)	73.4	75.0	71.7
ACE-inhibitors (%)	34.4	28.3	40.9
ARB (%)	38.5	45.7	31.1
<b>Diabetes medication</b>			
Insulin (%)	73.7	71.4	76.1
Metformin (%)	60.8	57.1	64.6
DPP4-i (%)	4.2	6.1	2.1
GLP1-RA (%)	2.1	0.0	4.3
SGLT2-i (%)	2.1	0.0	4.3
<b>Lipid-lowering (%)</b>	80.2	68.9	91.3
Statins (%)	79.5	80.6	78.6
Chol-transport binders (%)	6.8	3.2	9.5
Fibrates (%)	5.5	6.4	4.8
<b>Antidepressants (%)</b>	7.5	8.5	6.5
<b>Composite vascular complications (%)</b>	52.1	57.1	46.8
CVD (%)	11.5	12.2	10.6
Retinopathy (%)	32.1	37.2	26.8
Polyneuropathy (%)	33.3	30.2	36.6
<b>Baseline MOFs consumption mg/day</b>	77.4 (43.5-223)	75.4 (45.2-204)	83.9 (40-245)
<b>Alcohol-use (%)</b>	34.4	32.6	36.4
<b>Smoking (%)</b>			
Past	18.6	16.3	20.8
Current	4.1	4.1	4.2
<b>Physical-activity, ≥30 min/day (%)</b>			
0 days/week	13.6	14.3	12.8
1–4 days/week	39.6	26.2	33.3
5–7 days/week	55.6	59.5	51.3

SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; AER: albumin excretion rate; alb/creat: albumin/creatinine ratio in urine; eGFR: estimated glomerular filtration rate; TG: triglycerides; total-C: total cholesterol; HbA1c: glycated hemoglobin; sVCAM-1: soluble vascular cell adhesion molecule-1; sICAM-1: soluble intercellular cell adhesion molecule-1; vWF: von Willebrand Factor; RAS-I: renin-angiotensin system inhibitor; ARB: angiotensin receptor blocker; MOF: monomeric and oligomeric flavanols; Composite vascular complications: the presence of one or more microvascular or macrovascular complication on top of microalbuminuria; CVD: the presence of one or more macrovascular complication including: angina pectoris, myocard infarction, PTCA/PCI, transient ischemic attack, cerebrovascular event, peripheral arterial disease.

<sup>a</sup> Data are given as mean ± SD or as median (interquartile range).

complicated versus non-complicated T2D. It is important to stress that grape seed extracts can differ substantially in the number and mass of bioactive compounds, such as MOFs. As a result, comparison of the dosages and effects of different grape seed extract products is not meaningful. To monitor the food constituent and composition in our MOFs in every individual production batch, <sup>1</sup>Hydrogen-Nuclear Magnetic Resonance (<sup>1</sup>H NMR) fingerprinting and subsequent Principal Component Analysis (PCA) were used [39,40]. Our MOFs dosage and duration of intervention was based

on previous human intervention studies, showing favorable effects in healthy individuals at 100–300 mg daily [30,31].

#### 4.1. Stage of disease

Our study participants were older and had a long history of less well-controlled diabetes than the participants in most other studies [23,24]. The patients in the study by Kar et al. [24] had non-complicated T2D, whose treatment was restricted to lifestyle



**Table 2**  
Within-group changes during intervention.

	Baseline <sup>a</sup>	6 weeks <sup>a</sup>	3 months <sup>a</sup>	Delta baseline-3 months <sup>a</sup>	p-value <sup>b</sup>
<b>AER (mg/24 h)</b>					
Intervention	80 (27-120)	50 (20-88)	50 (28-120)	0 (-35-21)	0.41
Control	60 (18-120)	60 (20-125)	35 (20-125)	0 (-20-10)	0.91
<b>sICAM-1 (ng/ml)</b>					
Intervention	64.9 (43.8-113.6)		61.2 (39.4-103.4)	0 (-6.7-5.1)	0.85
Control	59.5 (35.8-90.4)		59.1 (34.9-83.9)	0 (-7.5-4.7)	0.59
<b>sVCAM-1 (ng/ml)</b>					
Intervention	726 (564-869)		726 (691-853)	-8 (-93-75)	0.70
Control	664 (530-895)		704 (539-916)	34 (-24-116)	0.06
<b>vWF (IU/ml)</b>					
Intervention	384 (264-449)		405 (300-472)	2 (-44-101)	0.42
Control	393 (286-492)		383 (302-514)	32 (-90-74)	0.89

AER: albumin excretion rate; sVCAM-1: soluble vascular cell adhesion molecule-1; sICAM-1: soluble intercellular cell adhesion molecule-1; vWF: von Willebrand Factor.

<sup>a</sup> Data are given as median (interquartile range).

<sup>b</sup> Within-group difference baseline-3 months, analyzed by Friedman-test when >2 time-points and by Wilcoxon Signed Rank test when 2 time-points.

**Table 3**  
Between-group differences in the evolution over time of AER (primary endpoint) and sICAM-1, sVCAM-1 and vWF (secondary endpoints).

	Unadjusted model <sup>a</sup>		Adjusted model <sup>b</sup>	
	Estimates (95%CI)	p-value	Estimates (95%CI)	p-value
AER	-0.02 (-0.23-0.20)	0.88	-0.04 (-0.27-0.19)	0.72
sICAM-1	-0.00 (-0.10-0.09)	0.95	-0.00 (-0.11-0.10)	0.95
sVCAM-1	-0.15 (-0.39-0.09)	0.21	-0.09 (-0.33-0.16)	0.48
vWF	0.14 (-0.25-0.52)	0.48	0.22 (-0.17-0.61)	0.26

AER: albumin excretion rate; sVCAM-1: soluble vascular cell adhesion molecule-1; sICAM-1: soluble intercellular cell adhesion molecule-1; vWF: von Willebrand Factor.

<sup>a</sup> Treatment\*time interaction of linear mixed-model on log-transformed data.

<sup>b</sup> Adjusted for sex, age and BMI.

advice and oral anti-diabetes medication. It is tempting to hypothesize that patients with a shorter duration of T2D, well-controlled diabetes, less medication, and less affected vascular health, will benefit more from a dietary intervention with MOFs than those with more complicated T2D, like in our study. This hypothesis is in line with the observations that vascular function, determined with the invasive fore-arm model, was improved by daily red wine consumption in healthy young women [41] but not in patients with T2D [42]. Notably, the majority of the RCTs in individuals without T2D in which MOFs had positive effects on vascular health, were conducted in healthy individuals without any cardiovascular risk factor [43,44], or in individuals with prehypertension and mild hypertension [45,46], mild to moderate hyperlipidemia [47], and metabolic syndrome [48]. Unlike all but one abovementioned studies, our patients had advanced vascular and endothelial damage.

4.2. Medication use

Most of our participants used multiple medications to treat diabetes, blood pressure, and dyslipidemia. Besides antihypertensives, also fibrates, SGLT2-i and GLP1 receptor agonists are reported to protect the microvasculature [11–13]. Although used by only a few participants of our study, these medications may provide an alternative explanation for inconsistent findings. If RAS-I and the other medications (partially) act through the same pathways as MOFs, untreated patients would have been required to test the MOFs effect. However, ethical considerations prevent withholding patients with microalbuminuria such treatment. In our patients, microalbuminuria has persisted despite medication, potentially resulting from non-compliance or therapy-resistant

microalbuminuria. We did not observe interactions between the effects of MOFs intake and the use of ACE-inhibitors or statins (data not shown). The groups on fibrates, SGLT2-I and GLP-1 receptor agonists were too small for meaningful analyses.

4.3. Strengths and limitations

One could argue that the short duration of the intervention is a limitation of our study. However, the duration and dosage were based on previous studies using the same MOFs compound, showing positive effects on cardiovascular risk factors in healthy smokers at 200 mg daily over a 2 month period [30,31]. Moreover, a three-month window to evaluate an effect fits perfectly in the T2D guidelines. We had anticipated an effect of 20% on treated microalbuminuria, aiming at a clinically relevant effect in this usual follow-up window. We cannot exclude that long-term use of MOFs reduces albuminuria, especially in the early stages of the disease. A small, non-significant decrease of AER was observed in the MOF treated group and in the placebo group. Limited power and a small placebo effect may have reduced our capability to observe an effect of MOF. Another limitation of this study is the use of surrogate markers of endothelial function, instead of direct measures of endothelial function like flow-mediated dilation. The strengths of our study are the double-blind, randomized placebo-controlled design and the relatively large sample size. We have performed one of the largest RCT's in the field of flavanol research. Moreover, patients were recruited from a primary, secondary as well as a tertiary health care center. In addition, we tested MOFs on top of habitual diet and in combination with medications used by almost all patients with T2D. Our pragmatic approach, therefore, reflects realistic circumstances. However, our results only apply to treated patients with T2D with microalbuminuria and relatively short follow-up compared to the recent LEADER trial analysis [8].

5. Conclusion

In conclusion, we did not find support for a beneficial effect on the urinary excretion of albumin and markers of vascular function of three months 200 mg MOFs from grape seeds daily, in the dietary management of patients with treated, long-term, T2D and microalbuminuria. The short follow-up, the advanced stage of vascular damage, and the simultaneous use of multiple medications may explain the neutral findings. Analyses of long-term, high dosages of MOFs treatment are required to determine whether using MOFs can affect microalbuminuria in patients with complicated T2D. Studies with MOFs in patients with uncomplicated T2D are needed to test if MOFs can prevent microalbuminuria.

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## Conflict of interest

The authors declare that they have no competing interests.

## Author contribution

Mardin Rashid: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Project administration. Adrie J.M. Verhoeven: Conceptualization, Resources, Writing - Review & Editing, Monique T. Mulder: Conceptualization, Resources, Writing - Review & Editing. Reinier Timman: Methodology, Formal analysis, Writing - Review & Editing. Behiye Ozcan: Investigation, Resources, Writing - Review & Editing. Yvonne van Beek-Nieuwland: Investigation, Resources, Writing - Review & Editing. Lei M. Chow: Investigation, Resources, Writing - Review & Editing. Roel J.J.M. van de Laar: Investigation, Resources, Writing - Review & Editing. Willem A. Dik: Investigation, Resources, Writing - Review & Editing. Eric J.G. Sijbrands: Conceptualization, Methodology, Writing - Review & Editing, Supervision, Funding acquisition. Kirsten A. Berk: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Supervision, Funding acquisition.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2021.09.038>.

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