Resveratrol treatment does not reduce arterial inflammation in males at risk of type 2 diabetes: a randomized crossover trial

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Eine Behandlung mit Resveratrol führt nicht zu einer Reduktion der arteriellen Entzündung bei Männern mit einem erhöhten Risiko für Typ-2-Diabetes: Eine randomisierte Crossover-Studie

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ABSTRACT

Purpose Resveratrol has shown promising anti-inflammatory effects in in vitro and animal studies. We aimed to investigate this effect on arterial inflammation in vivo.

Methods This was an additional analysis of a double-blind randomized crossover trial which included eight male subjects with decreased insulin sensitivity who underwent an ^{18}F-fluoroxyglucose (^{18}F-FDG) PET/CT after 34 days of placebo and resveratrol treatment (150 mg/day). ^{18}F-FDG uptake was analyzed in the carotid arteries and the aorta, adipose tissue regions, spleen, and bone marrow as measures for arterial and systemic inflammation. Maximum target-to-background ratios (TBR_{max}) were compared between resveratrol and placebo treatment with the non-parametric Wilcoxon signed-rank test. Median values are shown with their interquartile range.

Results Arterial ^{18}F-FDG uptake was non-significantly higher after resveratrol treatment (TBR_{max} all vessels 1.7 (1.6–1.7)) in comparison to placebo treatment (1.5 (1.4–1.6); $p = 0.050$). Only in visceral adipose tissue, the increase in ^{18}F-FDG uptake after resveratrol reached statistical significance ($p = 0.024$). Furthermore, CRP-levels were not significantly affected by resveratrol treatment ($p = 0.091$).

Conclusions Resveratrol failed to attenuate arterial or systemic inflammation as measured with ^{18}F-FDG PET in subjects at risk of developing type 2 diabetes. However, validation of these findings in larger human studies is needed.

ZUSAMMENFASSUNG

Ziel Resveratrol hat in In-vitro- und Tierstudien vielversprechende anti-inflammatorische Effekte gezeigt. Unser Ziel war es, diese Wirkung auf die arterielle Entzündung in vivo zu untersuchen.

Methoden Es handelte sich um eine zusätzliche Analyse einer randomisierten Doppelblind-Crossover-Studie, an der acht männliche Probanden mit verminderter Insulinsensitivität teilnahmen, die sich nach 34 Tagen Placebo- und Resvera-

trol-Behandlung (150 mg/Tag) einer ^{18}F-Fluoroxyglucose (^{18}F-FDG)-PET/CT unterzogen. Die ^{18}F-FDG-Aufnahme wurde in den Karotiden und der Aorta, Fettgeweberegionen, Milz und Knochenmark als Maß für die arterielle und systemische Entzündung analysiert. Die maximalen „Target-to-Background Ratios" (TBR_{max}) wurden zwischen Resveratrol- und Placebo-Behandlung mit dem nichtparametrischen Wilcoxon-Vorzeichen-Rang-Test verglichen. Die Medianwerte sind mit ihrem Interquartilsbereich angegeben.

Ergebnisse Die arterielle ^{18}F-FDG-Aufnahme war nach der Resveratrol-Behandlung nicht signifikant höher (TBR_{max} alle Gefäße 1,7 (1,6–1,7)) im Vergleich zur Placebo-Behandlung (1,5 (1,4–1,6); p = 0,050). Nur im viszeralen Fettgewebe war der Anstieg der ^{18}F-FDG-Aufnahme nach Resveratrol statistisch signifikant (p = 0,024). Auch die CRP-Werte wurden durch die Resveratrol-Behandlung nicht signifikant beeinflusst (p = 0,091).

Schlussfolgerungen Resveratrol konnte die mittels ^{18}F-FDG-PET gemessene arterielle oder systemische Entzündung bei Personen mit einem Risiko für die Entwicklung eines Typ-2-Diabetes nicht abschwächen. Allerdings ist eine Validierung dieser Ergebnisse in größeren Humanstudien erforderlich.

Introduction

Resveratrol (trans-3,4',5-trihydroxystilbene) is a natural constituent of berry fruits, peanuts, grapes and other plants. Resveratrol production is increased when the plant is stressed; for instance, during drought or infection [1]. It has been suggested that resveratrol acts as a xenohormetic compound, meaning that the resveratrol in these plants could affect the biology of animals consuming them. In this way, the presence of high levels of resveratrol may act as a warning sign for "tougher times ahead". This could explain the calorie-restricting and longevity effects of resveratrol in some animals, because a period with less food to go around would be unattractive to reproduce and therefore ageing is slowed to postpone reproduction [1]. In concurrence, resveratrol appears to affect aging-related diseases, such as cancer and cardiovascular disease [1, 2, 3]. Among others, resveratrol is believed to influence atherosclerosis via several mechanisms, such as decreasing lipid peroxidation, lipid phagocytosis, platelet aggregation, oxidative stress, hypertension, insulin resistance, and by influencing endothelial function [1, 2, 3, 4]. Animal models have confirmed these beneficial effects. Resveratrol attenuated plaque formation in apolipoprotein-E (apoE) knockout mice, a well-known atherosclerosis model [5], as well as in a double knockout model for apoE and low-density lipoprotein receptor (LDLR) [6] and in apoE knockout mice with additional angiotensin-II administration [7]. In rabbits fed a high-cholesterol diet, addition of resveratrol delayed atherosclerotic plaque progression independent of lipid levels [8]. Moreover, macrophage count in the plaques was lower, indicating reduced arterial inflammation [7].

Despite the promising findings in animal studies, relatively few and mainly small studies on the effects of resveratrol in humans have been published so far [9, 10]. To our knowledge, various studies have investigated the effects of resveratrol on cardiovascular risk factors but none on arterial inflammation. Both systemic inflammation and inflammation of the arterial wall have been related to an increased incidence of cardiovascular events, like myocardial infarction, abdominal aortic aneurysm rupture or stroke [11, 12, 13, 14]. ^{18}F-fluordeoxyglucose (^{18}F-FDG) positron emission tomography (PET) is nowadays frequently used to image arterial wall inflammation *in vivo*, and validation studies with carotid endarterectomy and aortic aneurysm specimens have confirmed ^{18}F-FDG uptake to correlate with the degree of macrophage infiltration [14, 15]. Arterial wall ^{18}F-FDG PET has therefore been used as a non-invasive secondary endpoint in several interventional studies [16, 17, 18, 19, 20].

In addition, increased ^{18}F-FDG uptake in the spleen, bone marrow and different adipose tissues has been correlated to higher arterial uptake and to recent and future cardiovascular events as well [21, 22, 23].

Here, we report the results of an exploratory analysis as part of a randomized double-blind crossover trial evaluating the effect of resveratrol on arterial inflammation. We hypothesized that the use of resveratrol would reduce arterial inflammation as measured with ^{18}F-FDG PET.

Methods

Study design

This is an additional analysis of the subgroup of subjects who underwent a ^{18}F-FDG PET/CT within the context of a recently published double-blind crossover design study investigating the effects of resveratrol in subjects at risk of developing type 2 diabetes [24]. The local medical ethics committee of the academic hospital and university of Maastricht (aZM/UM) approved this project and all participants signed an informed consent form before any study procedures were conducted. All study procedures were performed at Maastricht University and Medical Center and were in compliance with the Declaration of Helsinki. The trial was registered on clinicaltrials.gov (study ID: NCT02129595, date of registration: 02/05/2014).

The original study of de Ligt et al. included 15 subjects of whom eight underwent additional ^{18}F-FDG PET/CT scans [24]. All subjects received both placebo and trans-resveratrol treatment of 75 mg (99.9 % purity) 2 times daily for 34 days. An enrolment diagram is included as supplementary data 1. The order of treatment was randomized by an independent researcher using www.randomization.com to result in an equal distribution in treatment order among the total of 15 subjects. After the first treatment period, subjects switched treatments with a washout period of at least 30 days in between. Both the capsules containing resveratrol and placebo were provided by DSM Nutritional Products Ltd. Containers were marked sequentially to blind both the subject and the

▶ **Table 1** In- and exclusion criteria.

Inclusion	Exclusion
▪ Male ▪ Age 40–70 years ▪ Has first-degree relative(s) with diabetes type 2 ▪ Overweight (BMI 27–35 kg/m^2) ▪ Sedentary lifestyle (no physically active job, exercises less than 2 hours per week) ▪ Stable dietary habits (no weight change > 5 kg in the last three months) ▪ Insulin-resistant (as determined by a glucose clearance rate of < 350 ml/kg/min using the Oral Glucose Insulin Sensitivity Model ($OGIS_{120}$) based on a 2-hour oral glucose tolerance test) ▪ Willingness to abstain from resveratrol-containing food products ▪ Study inclusion is approved by the study related medical doctor based on screening data.	▪ Use of anticoagulants ▪ Uncontrolled hypertension ▪ Anemia (Hb < 7.8 mmol/L) ▪ Type 2 diabetes diagnosis ▪ HbA1c > 6.5 % ▪ Use of medication known to interfere with glucose homeostasis/metabolism ▪ Rest-state ECG-abnormalities may be reason for exclusion based on the judgment of the study medical doctor. ▪ Current alcohol consumption > 20 g/day (≈ 2.5 unit/day)

BMI: body mass index; ECG: electrocardiogram; Hb: hemoglobin; HbA1c: hemoglobin A1c.

researchers. Only at the end of the study, researchers were informed of the treatment order.

Subjects

Between April 2014 and October 2016, volunteers were screened according to the in- and exclusion criteria shown in ▶ **Table 1**. Participants were considered to have impaired insulin-sensitivity when they had a glucose clearance rate of < 350 ml/kg/min according to the Oral Glucose Insulin Sensitivity Model ($OGIS_{120}$) based on a standardized 2-hour oral glucose tolerance test (OGTT). A subset of eight subjects underwent a PET/CT twice – once at the end of each treatment period at day 34 to study brown adipose tissue (BAT) activation. In the current analysis, we used both scans of these subjects, and hence, subjects served as their own control. During the study, participants had to abstain from resveratrol containing foods to prevent resveratrol from dietary intake influencing the results. No adverse events related to the treatment were reported. For details see de Ligt et al. [24].

Blood analyses

Fasting resveratrol (free + conjugated) levels and a metabolite of resveratrol (dihydroxyresveratrol (DHR)) were determined on day 7, 14, 21 and 30 to control for adequate intake of the resveratrol capsules and adherence to dietary restrictions during the washout and placebo period. Routine clinical chemistry including renal function, liver enzymes, glucose, HbA1c and insulin was determined on day 0 and 30 of each treatment period. Additionally, C-reactive protein (CRP)-levels were determined on day 30 of both treatment periods. All blood samples were analyzed on the Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany), except for the HbA1c (D100, Bio-Rad Laboratories, Hercules, United States), insulin (Immulite XPi, Siemens Healthcare, Erlangen, Germany), and CRP (Cobas 8000, Roche Diagnostics).

PET protocol

^{18}F-FDG PET/CT scans were performed to study BAT activation. Hence, participants were scanned after a dedicated cooling protocol, required to activate BAT [24]. A standard fixed dose of approximately 75 MBq ^{18}F-FDG was injected one hour before scanning. Static PET images were acquired (6–7 bed positions, 6 min per bed position) using a Gemini TF PET/CT (Philips Healthcare, Eindhoven, The Netherlands) after an overnight fast and confirmation of appropriate serum glucose levels. Subjects were scanned using a low-dose non-contrast whole-body CT protocol (120 kVp, 30 mAs) for attenuation correction and co-localization of the PET signal with the anatomy. Radiation dose was calculated to be ~5.85 mSv for each participant in total.

Image analysis

Image analysis was performed on a dedicated commercially available workstation (Extended Brilliance Workspace V4.5.3.40140; Philips Healthcare, Eindhoven, The Netherlands). The researchers were blinded for treatment at the time of evaluation of the scans and only received the de-blinding code after all scans were analyzed.

Regions of interest (ROIs) were manually drawn to encompass the whole vessel of the common carotid arteries and the aorta. The latter was divided accordingly; ascending aorta, aortic arch, thoracic descending aorta (till the diaphragm) and abdominal aorta (till the iliac bifurcation or as low as sufficiently imaged). The ^{18}F-FDG uptake within each ROI was represented by the mean and maximum standardized uptake value (SUV_{mean} and SUV_{max}), respectively. This measurement represents the activity per ROI corrected for decay (dependent on injected dose and time after injection) and body weight of the subject. The SUV_{mean} and SUV_{max} of all ROIs were corrected for blood pool activity by calculating target-to-background ratios (TBR). Hereto, arterial SUVs were divided by the average SUV_{mean} of at least three standardized ROIs (4 mm) placed in the lumen of the jugular vein (TBRs of the carotid arteries) and of the superior cava vein (for all aortic TBRs). Correction of the SUV_{mean} and SUV_{max}, thusly resulted in the corresponding TBR_{mean} and TBR_{max}. For the carotid arteries, only the highest mean TBR_{max} value of both carotid arteries was used for analysis.

Additional standardized ROIs were placed to quantify activity in bone marrow (20 mm ROIs placed in the center of each thoracic and lumbar vertebra), spleen (20 mm ROIs encompassing all transversal slices), visceral adipose tissue (VAT; 10 mm ROIs placed in intra-peritoneal fat at the level of the umbilicus) and subcutaneous adipose tissue (SAT; 10 mm ROIs placed in the nuchal subcutaneous fat). TBRs were calculated by dividing corresponding mean and maximal SUVs by the $_{mean}SUV_{mean}$ of the superior cava vein (for spleen, bone marrow and VAT TBRs) or of the jugular vein (for TBRs in the nuchal SAT).

▶ **Table 2** Subject characteristics and laboratory tests after placebo and resveratrol treatment.

	Placebo	Resveratrol	p-value
^{18}F-FDG activity (MBq) [a]	75 (73–78)	76 (73–79)	0.575
Core temperature (°C) [a,b]	36.8 (36.4–37.0)	36.8 (36.7–36.9)	0.917
Weight (kg)	90 (86–95)	91 (87–95)	0.575
MAP (mmHg)	103 (97–108)	105 (99–113)	0.207
Resveratrol (ng/mL) [c]	ND	273 (194–312)	NA
DHR (ng/mL) [c]	ND	674 (365–926)	NA
Glucose (mmol/L)	5.8 (5.4–6.1)	5.5 (5.3–6.1)	1.000
Insulin (pmol/L)	68 (33–99)	78 (40–89)	0.575
HbA1c (%)	5.8 (5.6–5.8)	5.8 (5.5–5.9)	0.416
Cholesterol (mmol/L)	5.3 (4.6–6.1)	5.5 (5.1–6.0)	0.105
LDL (mmol/L)	3.3 (2.9–4.1)	3.4 (2.7–4.1)	0.673
CRP (mg/L)	2.5 (1.0–3.2)	1.6 (0.6–3.1)	0.091
$_{mean}SUV_{mean}$ blood pool JV [a]	1.5 (1.3–1.6)	1.6 (1.4–1.7)	0.553
$_{mean}SUV_{mean}$ blood pool VCS [a]	1.7 (1.4–1.9)	1.5 (1.5–1.6)	0.206

Median values are given for n = 8 with interquartile range depicted as (Q1-Q3) unless stated otherwise. [a] These results are from day 34 as opposed to day 30 for the remaining results. [b] Core temperature during cold stimulation (n = 6 pairs). [c] The average plasma levels of the four weekly measurements per patient were used to determine the group median. Abbreviations: MAP: mean arterial pressure; ND: not detectable; NA: not applicable; JV: jugular vein, VCS: vena cava superior.

ROIs were excluded from further analysis in case of spill-in of activity from adjacent structures, such as active BAT, or when motion-artefacts prevented the drawing of representable ROIs.

Statistical analysis

Continuous data are described using median and interquartile range (IQR), which is depicted as the values for the first (25 %) to the third (75 %) quartile (Q1-Q3). Baseline characteristics, laboratory tests and ^{18}F-FDG-uptake were compared between treatment periods, using the non-parametric Wilcoxon signed-rank test and the Friedman test was used to study differences of resveratrol (metabolite) levels between the time points within the same treatment period. Statistical significance was defined at the 95 %-confidence level ($p < 0.05$). All statistical analyses were performed with SPSS Statistics (version 24.0 for Macintosh, released 2016; IBM Corp, Armonk, NY, USA).

Results

Subject characteristics and blood analyses

Eight male participants with a median age of 66 (53–68) years, a median BMI of 28.9 (27.8–29.6) kg/m^2 and a median glucose clearance rate of 334 (305–342) mg/dL, as determined by $OGIS_{120}$, were included. No clinically or statistically significant differences in baseline characteristics, injected ^{18}F-FDG activity, or plasma lipids and markers of glucose metabolism were found between treatments (see ▶ **Table 2** and de Ligt et al. for more details [24]).

In all subjects, resveratrol and DHR levels were determined weekly to verify adequate resveratrol supplement intake, metabolism and adherence to dietary restrictions. During the placebo period, these levels were below the detection limit, whereas under resveratrol treatment, they were detectable. The levels of resveratrol, DHR and their ratio did not differ statistically significant between the four time points within the resveratrol period ($p = 0.175$, $p = 0.226$ and $p = 0.092$, for resveratrol, DHR and DHR/resveratrol ratio, respectively). The median of the group's average resveratrol and DHR levels for the entire period are presented in ▶ **Table 2**.

CRP-levels decreased in six out of eight participants, stayed unchanged in one participant and increased in another. The median CRP after placebo treatment was 2.5 (1.0–3.2) mg/L and 1.6 (0.6–3.1) mg/L after resveratrol treatment ($p = 0.091$).

PET/CT image quality

In all subjects (n = 8), image quality of both scans was sufficient to include all aortic regions in the analysis. However, due to motion artefacts in the neck region, ^{18}F-FDG uptake in the carotid arteries could not be analyzed in three scans belonging to two participants. In one participant only the right carotid artery could be used in the analysis due to spill-in in the left carotid artery. Results for the carotid arteries are therefore based on n = 6.

▶ **Table 3** TBR_{max} after placebo and resveratrol treatment.

	Placebo	Resveratrol	p-value
Carotid arteries [a, b]	1.2 (1.2–1.5)	1.4 (1.3–1.9)	0.058
Ascending aorta	1.7 (1.5–1.8)	1.7 (1.7–1.7)	0.524
Aortic arch	1.5 (1.3–1.8)	1.6 (1.6–1.7)	0.140
Thoracic aorta	1.6 (1.5–1.8)	1.8 (1.6–1.8)	0.202
Abdominal aorta	1.5 (1.5–1.7)	1.6 (1.5–1.7)	0.216
All vessels	1.5 (1.4–1.6)	1.7 (1.6–1.7)	0.050
Spleen	1.5 (1.3–1.6)	1.6 (1.5–1.7)	0.201
Bone marrow	1.6 (1.3–1.9)	1.6 (1.5–1.9)	0.098
SAT	0.5 (0.4–0.5)	0.3 (0.3–0.5)	0.339
VAT	0.3 (0.2–0.5)	0.5 (0.4–0.7)	0.024

Median values are given for n = 8 with interquartile range depicted as (Q1-Q3) unless stated otherwise. [a] n = 6 pairs. [b] The highest value from both carotid arteries was selected for further analysis.

Effect of resveratrol on arterial inflammatory activity

Differences in SUV_{mean} and SUV_{max} between the interventions were small and did not reach statistical significance in any vascular territory (a table of the SUV values is given in supplementary data 2). In accordance, only slight differences in TBRs were observed in general (▶ **Table 3** and ▶ **Fig. 1**). It is noteworthy that TBRs were not only 'not lower' after treatment with resveratrol in comparison to placebo treatment but actually tended to be higher (TBR_{max} all vessels 1.7 (1.6–1.7) vs. 1.5 (1.4–1.6)), although this was not statistically significant (p = 0.050). A figure of an exemplary PET/CT scan is given in ▶ **Fig. 2**.

Effect of resveratrol on ^{18}F-FDG uptake in the spleen, the bone marrow and white adipose tissues

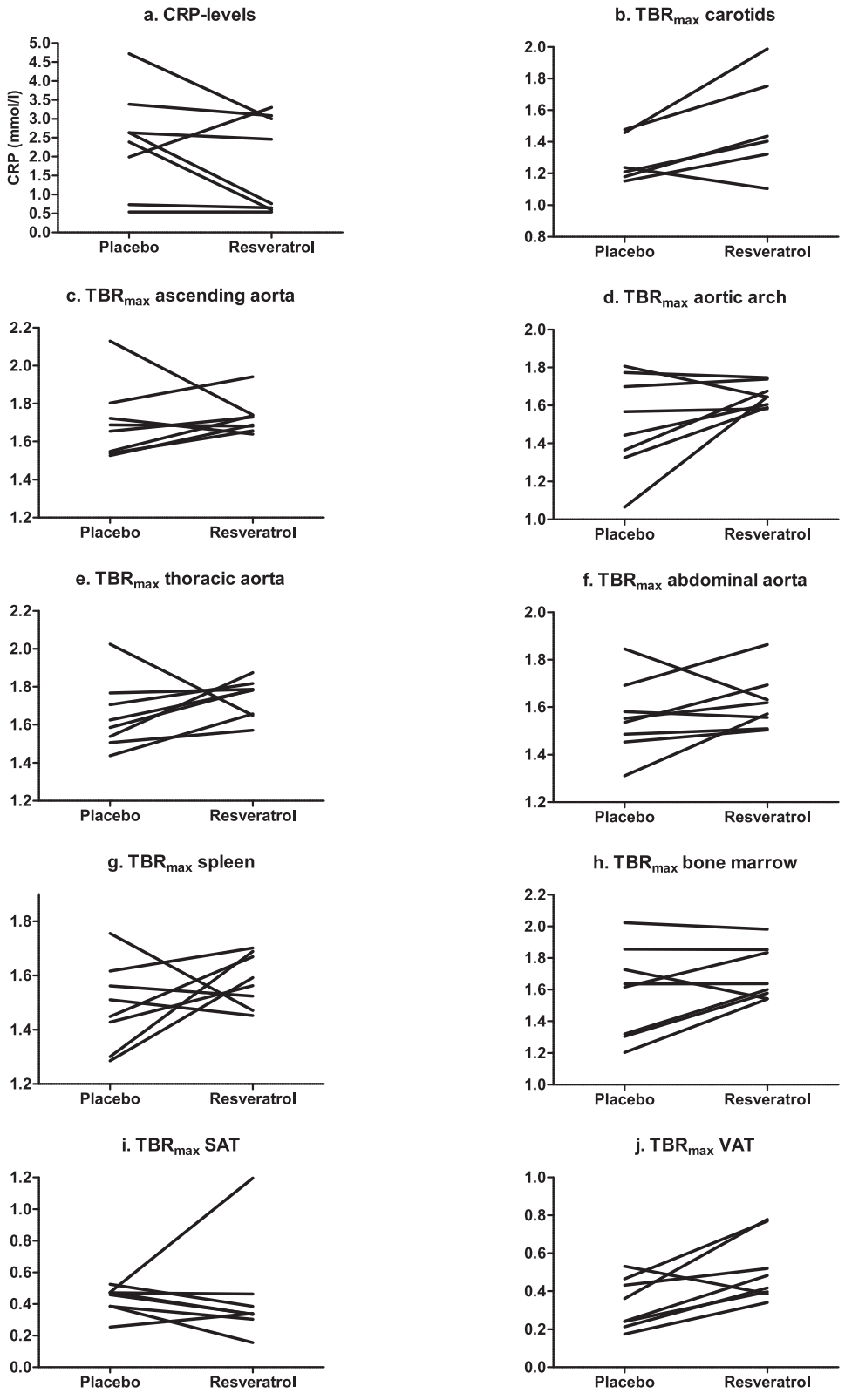
Similar to the arterial TBRs, TBRs of the spleen, bone marrow and VAT tended to be higher after treatment with resveratrol compared to those after placebo (▶ **Table 3** and ▶ **Fig. 1**). This difference was only significant for VAT (p = 0.024 for TBR_{max}). Contrary to all previous findings, SAT showed a slight decrease in TBR_{max}.

Discussion

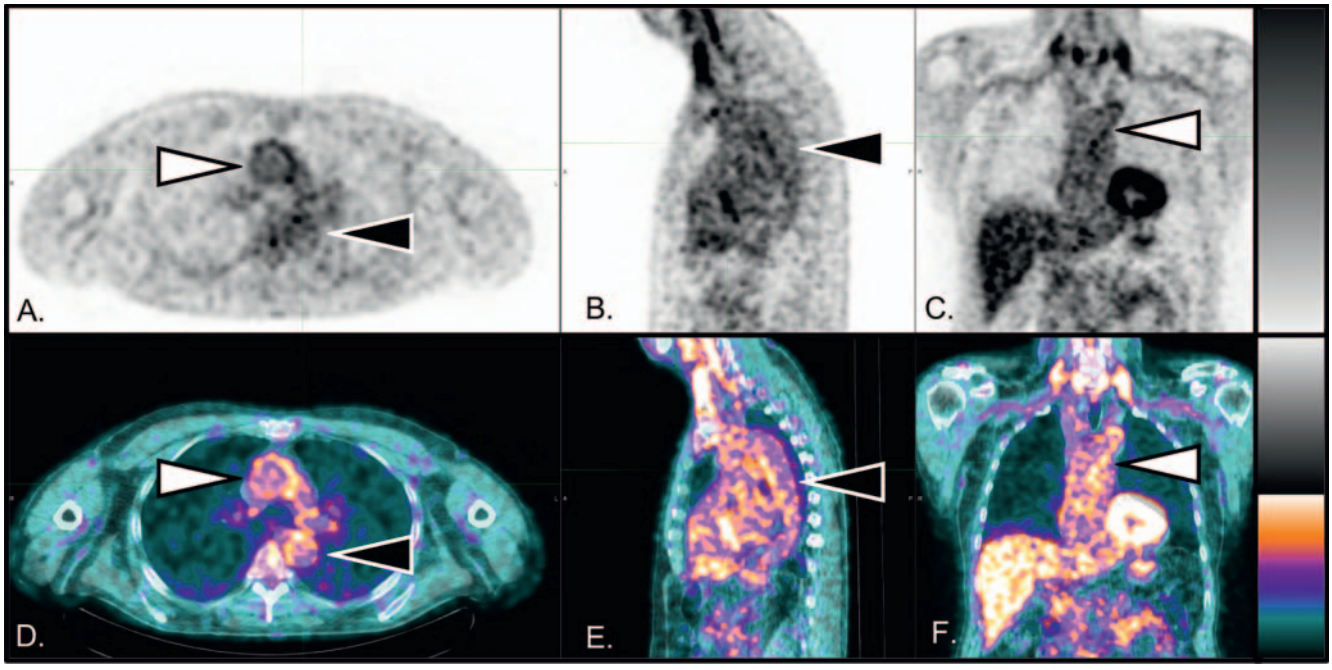
Resveratrol treatment did not result in lower arterial uptake of ^{18}F-FDG in subjects at risk of developing type 2 diabetes in comparison to placebo treatment. This implies that there was no significant effect on arterial inflammation. Although animal studies have been promising in this regard [5, 6, 7, 8], our findings rather seem to be in line with previously published studies questioning the anti-inflammatory effects of resveratrol in humans [10, 25].

Arterial wall ^{18}F-FDG uptake has been correlated to plaque macrophage content [15], which in its turn has been related to the risk of developing subsequent cardiovascular events [11, 12, 13]. Decreasing both local and systemic arterial inflammation has therefore been an important target in cardiovascular research [11, 26]. ^{18}F-FDG has been used to establish the anti-inflammatory effects of several treatment options, including lipid-lowering therapy [16], life style measures [17] and bariatric surgery [27]. It would be cause for excitement if similar effects could be accomplished with a food supplement, like resveratrol. In apoE knockout mice, resveratrol was able to reduce macrophage content in atherosclerotic plaques [7], and reduced plaque progression was established in several other animal models as well [5, 6, 8]. However, resveratrol has shown a myriad of effects in animals, but has only recently been used in small human trials. Despite a few studies showing positive effects of resveratrol, for instance on insulin-sensitivity [28] and blood lipid levels [29], an evident number of studies indicated no effect of resveratrol [25]. Other studies only observed positive effects in specific subgroups of patients [30, 31, 32] or at high doses of resveratrol [32, 33]. Our findings may be in line with these disappointing results in humans.

Remarkably, we found higher TBR values after resveratrol treatment in comparison to the placebo treatment with p-values near statistical significance in the carotid arteries and when all vessels were combined. Although the consequent increase in TBR after resveratrol could suggest an actual detrimental effect of resveratrol on arterial inflammation, we consider this conclusion unlikely, since evidence for such an effect is scarce in the literature [1, 2, 3, 4, 34]. To our knowledge, only one study using rabbits fed an high-cholesterol diet found atherosclerotic lesions to be increased after resveratrol treatment [34]. However, this finding may be affected by the control group receiving a daily dose of 95 % ethanol of 0.05 ml/kg [34]. We therefore mainly consider the trend towards higher TBRs after resveratrol treatment as an indicator that a larger sample size would not have resulted in a statistically significant decrease in ^{18}F-FDG uptake after resveratrol treatment in this study design. However, it is possible that a carry-over effect explains the lower ^{18}F-FDG uptake during the placebo period which was more often the second period (6/8 subjects), even though statistical analysis showed no significant peri-



▶ **Fig. 1** Individual differences in CRP-level and TBR_{max} between placebo and resveratrol treatment are shown. Results are based on n = 8 with the exception of the TBR_{max} of the carotid arteries (n = 6). Although CRP-levels decreased in 6 out of 8 subjects, TBR_{max} increased in 4 to 7 out of 8 subjects in the arterial territories (5 out of 6 in the carotid arteries), the spleen, bone marrow and VAT. In SAT, TBR_{max} decreased in 5 out of 8. These graphs were created using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA).



▶ **Fig. 2** This figure shows an exemplary PET/CT-scan of a subject. In the top row (panel **A–C**), the PET-only images are shown. The bottem row (panel **D–F**) shows the fusion images. The white arrow heads point to the ascending aorta in the transversal plane (panel **A** and **D**) and in the coronal plane (**C** and **F**). The black arrowheads indicate the descending aorta in the transversal plane (panel **A** and **D**) and in the sagittal plane (panel **B** and **E**). The bars to the right indicate signal intensity; the colors that are highest on the bar indicate the highest signal. From the top down, these bars represent PET-signal as measured in SUV for the PET-only images, Houndsfield Units for the CT and again SUV for the PET-data in the PET/CT fusion image. In both the ascending and descending aorta, there is a high PET-signal in the arterial wall; most outspoken in the left-side of the ascending aorta and at the ventral of the descending aorta. In addition, there is a physiological high uptake in the myocardium and liver, and high uptake in the sternocleidomastoid muscles in the neck, probably due to motion.

od-effect. It is possible that the effects of resveratrol are slower than accounted for in this study and this is a major reason to plead for studies with longer treatment periods and a larger sample size.

A critical point in resveratrol research in humans in general is the low bioavailability of dietary resveratrol; surreal amounts of food products naturally containing resveratrol, such as red wine, would have to be consumed to achieve an intake equivalent to that used in (animal) studies [9, 35]. In a cohort study of Italian community-dwelling elderly, resveratrol metabolites in urine did not correlate with all-cause mortality, cardiovascular disease or cancer incidence, nor did it correlate with markers of inflammation, such as CRP [36]. It therefore has to be considered that resveratrol, in the amounts derived from a Western diet, does not substantially influence health status or life expectancy in humans [36]. Nonetheless, it is possible that resveratrol in higher than naturally occurring concentrations is beneficial, and it could be questioned whether the dosage as applied in the current study and/or the duration of treatment was sufficient. This question is underlined by the non-significantly lower CRP-levels in our population after resveratrol treatment and the findings of our main study [24]; despite a lack of effect on insulin sensitivity, intrahepatic lipid accumulation or BAT activation, *ex vivo* muscle mitochondrial function was improved in the main study [24].

The appropriate resveratrol dosage in human trials remains a topic of debate. In human trials, dosage ranges between 10 mg – 5 g have been used with ambiguous results [2, 3]. The regime chosen in this study, 150 mg/day for >30 days, was based on previous experience in our center. This treatment significantly reduced resting metabolic rate, systolic blood pressure, intrahepatic lipid content, circulating glucose, triglycerides, and inflammation markers, in healthy obese subjects [28], but was ineffective in subjects with type 2 diabetes [37]. Other studies have found positive results with dosages as low as 10 mg/day [38].

In addition to the effective dosage of resveratrol, the adequate duration of treatment has also not been established and is still a matter of debate. In previously published animal studies, atherosclerosis prone rodents were treated with resveratrol for at least 6 weeks [5, 6, 7, 8]. A similar period in humans would translate to ~4,5 years, depending on life stage [39]. Additionally, most studies using arterial ^{18}F-FDG uptake as an outcome measure in trials with interventions other than resveratrol adhered to a treatment period of at least 12 weeks before the post-treatment ^{18}F-FDG PET scan [16, 18, 19, 20]. However, there has been at least one study, showing significant effects of statin therapy on arterial ^{18}F-FDG uptake in humans after just 4 weeks of treatment [40].

Another uncertainty is the timing of resveratrol treatment. It is possible that resveratrol is more effective in a different stage of atherosclerosis. Our study subjects were not selected based on known atherosclerosis, but did have a significant number of cardiovascular risk factors; all participants were male, middle-aged, and obese, and had confirmed decreased insulin-sensitivity. In addition,

their baseline evaluation revealed hypertension (n = 6) and/or dyslipidemia (n = 7) in the majority of subjects even though none of these diseases had been previously diagnosed. Furthermore, in various subjects a high ^{18}F-FDG-uptake in the arterial wall could be established visually as can be seen in ▶ **Fig. 2**. In addition, calcifications were also common in this population, mainly in the aorta. Previous studies have used arterial ^{18}F-FDG as a secondary outcome measure in medical and life style intervention trials in similar asymptomatic patients [16, 17, 18, 40]. However, it is possible that resveratrol is only effective in advanced and highly inflamed atherosclerosis. Although, it has also been suggested that resveratrol may show the largest beneficial effects even before the development of fatty streaks and thus in young adults [5].

Besides the uncertainty of which population would benefit (most) from resveratrol treatment at which time point and from which dosage and duration in human research in general, the following limitation must be stated for this study in specific; the current study was designed to depict changes in BAT activity. Therefore, timing and dosage of ^{18}F-FDG was not optimized for imaging ^{18}F-FDG uptake in the arterial wall. However, PET and cooling protocols did not differ between the scans after placebo and resveratrol treatment and therefore should not have significantly impacted our findings. In addition, we only compared ^{18}F-FDG uptake after treatments. Measuring a difference in TBRs before and after each treatment might have corrected for a possible period-effect.

Another limitation was the small sample size. A larger sample size might confirm (or dismiss) the trend towards lower CRP-levels after resveratrol. Notwithstanding, we chose to use the data from a recent study on the effect of BAT out of practical and ethical considerations. This analysis should therefore be considered as exploratory.

Conclusions

In this exploratory analysis, we did not find a decrease of arterial ^{18}F-FDG-uptake after a 34-day treatment with 150 mg/day resveratrol in males at risk of developing type 2 diabetes. Neither did we observe a significant reduction of CRP-levels or the systemic inflammatory activity of the spleen and bone marrow. This might indicate a lack of anti-inflammatory effect of resveratrol on arterial inflammation. However, validation of these findings in a study using a higher dosage of, and/or a longer treatment with resveratrol and/or a population with advanced atherosclerosis is needed to rule out any beneficial effect, ideally including histological confirmation of inflammation.

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Conflict of Interest

Disclosures: Joachim E. Wildberger receives institutional grants from Agfa, Morstel, Belgium; Bayer Healthcare, Berlin, Germany; GE, Chicago, Illinois; Optimed, Ettlingen, Germany; Philips Healthcare, Best, the Netherlands; Siemens Healthineers, Forchheim, Germany, and personal fees from the speaker's bureau of Bayer Healthcare, Berlin, Germany and Siemens Healthineers, Forchheim, Germany. Ellen Boswijk, Marlies de Ligt, Marie-Fleur J. Habets, Alma M.A. Mingels, Wouter D. van Marken Lichtenbelt, Felix M. Mottaghy, Patrick Schrauwen and Jan Bucerius, have no relationships with industry currently or within the last two years. Conflicts of interest: All authors declare that they have no conflict of interest regarding the research presented in this manuscript.

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