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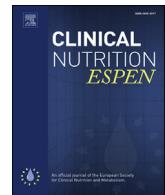


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Original article

Diet-induced weight loss and markers of endothelial dysfunction and inflammation in treated patients with type 2 diabetes



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SUMMARY

Background & aims: Overweight and obesity increase cardiovascular mortality in patients with type 2 diabetes (T2D). In a recent trial, however, diet-induced weight loss did not reduce the cardiovascular risk of patients with T2D, possibly due to the parallel intensive medical treatment. We investigated the effect of diet-induced weight loss on cardiovascular risk factors in overweight and obese patients with T2D, and whether this effect was influenced by the use of statins, ACE inhibitors, metformin and duration of T2D.

Methods: Patients with T2D and BMI >27 were subjected to an energy-restricted diet during 4 months. Before and after intervention, plasma levels of sICAM-1, sVCAM-1, hsCRP, vWF and classical biomarkers were measured. The association of the change in biomarker levels with medication use and T2D history, corrected for age, sex and change in insulin dose, was tested by matched linear regression analyses.

Results: In 131 patients, the diet resulted in weight loss of 10.2 kg (95%CI 9.2, 11.3; $p < 0.001$), improved median levels of HbA1c (-7.0 mmol/mol (95%CI $-8.5, -5.0$); $p < 0.001$), LDL cholesterol (-0.2 mmol/L (95%CI $-0.4, -0.1$); $p < 0.001$), sICAM-1 (-22.4 ng/mL (95%CI $-37.1, -8.7$); $p = 0.001$), vWF (-3.9 IU/mL (95%CI $-6.4, -1.4$); $p = 0.003$) and hs-CRP (-0.6 mg/L (95%CI $-1.2, -0.2$); $p = 0.007$), but did not affect sVCAM-1 levels (1.6 ng/mL (95%CI $-41.5, 48.6$); $p = 0.949$). Duration of T2D and medical treatment were not associated with these effects, except for an association between statin use and change in sVCAM-1, where statin users improved more.

Conclusion: Diet-induced weight loss reduced the levels of biomarkers of endothelial dysfunction and inflammation in overweight and obese patients with T2D independently of medication use and T2D duration. Even on intensive medical drug treatment as well as after a long history of T2D, patients may still profit from diet-induced weight reduction.

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1. Introduction

Cardiovascular disease is a feared complication of type 2 diabetes (T2D), as it is often fatal in persons with T2D [1]. Overweight and obesity enhance cardiovascular mortality in T2D [2]. In early asymptomatic stages, the insulin resistance typical for T2D has already detrimental effects on endothelial function [3]. This endothelial dysfunction is characterized by decreased vasodilation, increased vasoconstriction, insufficient or excessive angiogenesis, reduced barrier function, pro-coagulant activity, and a pro-inflammatory status, which are all associated with cardiovascular disease [4]. The soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1) and von

Abbreviations: ACE inhibitor, angiotensin-converting enzyme inhibitor; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; T2D, diabetes mellitus type 2; VLCD, very low-calorie diet; vWF, von Willebrand factor.

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Willebrand factor (vWF) are biomarkers of endothelial function. These markers are elevated in patients with T2D and have been linked to vascular complications and mortality in T2D [3,5–9]. The systemic inflammation marker high-sensitivity C-reactive protein (hs-CRP) is also associated with endothelial dysfunction, atherosclerosis as well as visceral adipose tissue mass [10,11]. The hs-CRP levels are clinically used to stratify cardiovascular risk [12].

Moderate intentional weight loss in overweight persons with and without T2D improves insulin sensitivity, blood pressure, lipid profile, endothelial function, and alleviates the pro-inflammatory state [13–19]. However, weight loss resulting from a lifestyle intervention did not reduce the cardiovascular risk of patients with T2D in the Look AHEAD trial [20]. The use of well-established, cardio-protective drugs such as statins, angiotensin-converting enzyme (ACE) inhibitors and metformin, which are commonly prescribed in T2D, might have reduced the contrast between the groups in this study. Alternatively, pathological changes might have become irreversible after a long history of T2D.

In this study, we have investigated the effect of diet-induced weight loss on biomarkers of endothelial dysfunction and inflammation in overweight and obese patients with T2D. Subsequently, we determined the associations between the change in biomarkers and the use of cardio-protective medication and the time since diagnosis of T2D.

2. Materials and methods

The present study is a pragmatic before-after study in the run-in phase of the Prevention of Weight Regain (POWER) trial of which the protocol has been published previously [21]. This study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam (reference number MEC-2009-143/NL26508.078.09), in compliance with the Helsinki Declaration. All participants provided informed consent.

2.1. Study population

Overweight and obese T2D patients ($\text{BMI} > 27 \text{ kg/m}^2$) aged 18–75 years were recruited from the outpatient diabetes clinic of the Erasmus Medical Center, Rotterdam from 2010 to 2013. Exclusion criteria were pregnancy, lactation, severe psychiatric problems, significant cardiac arrhythmias, unstable angina, decompensated congestive heart failure, major organ system failure, untreated hypothyroidism, and end-stage renal disease, and a myocardial infarction, cerebrovascular accident or major surgery during the previous 3 months. For all participants in the present study, antidiabetic and cardio protective medication was constant for at least 3 months prior to the intervention.

2.2. Diet intervention and data collection

The participants started with a very low-calorie diet (VLCD) of 750 kcal per day for a period of 8 weeks. The VLCD consisted of 2 commercially available meal replacements (Glucerna SR[®]) per day plus 75 g of lean meat, 150 ml of skimmed milk, and low-carbohydrate vegetables ad libitum. Subsequently, participants followed a low-calorie diet of 1100–1300 kcal/day for another 12 weeks. After 20 weeks, the participants used a 1300 kcal/day diet based on national health recommendations. During the diet program, 60 min of daily exercise was encouraged. To reduce the risk of hypoglycemia, the doses of sulfonylurea-derivatives and insulin were lowered before start of the dietary intervention but after baseline measurements. During the dietary intervention, insulin dose was adjusted based on glucose levels. Since insulin was adjusted during the intervention, we did not analyze the

interaction of insulin use with the diet-effect, but in all the analyses diet-induced effects were corrected for the insulin change during the intervention. The doses of metformin, statins and ACE inhibitors were maintained during the intervention.

Plasma samples were obtained after an overnight fast, and samples were stored at -80°C until further analysis. Of the 206 patients, who followed the dietary intervention, blood samples were obtained before as well as immediately after the dietary intervention of 131 patients. To enable participants to serve as their own control, only these 131 patients were enrolled in the current study. The other 75 patients did not differ significantly from the enrolled patients with respect to age, sex, baseline weight, baseline HbA1_c or diabetes complications (data not shown). We recorded demographic variables, duration of diabetes, smoking status and medication use, and measured weight (kg), waist circumference (cm) and blood pressure of all participants before and after the dietary intervention. The combined use of statins, ACE inhibitors and metformin was defined as 'maximum treatment'. Glycated hemoglobin (HbA1_c (mmol/mol)), fasting glucose (mmol/l) and plasma lipids (mmol/l) were measured using routine lab techniques. Physical activity was measured using the Short Questionnaire to Assess Health Enhancing Physical Activity (SQUASH) [22]. The study was monitored and documented with the trial management system OpenClinica (Waltham, MA).

2.3. Measurement of biomarkers of endothelial dysfunction and inflammation

Biomarker levels were measured in plasma by enzyme-linked immunosorbent assays (ELISA). sICAM-1, sVCAM-1 and hs-CRP were measured using DuoSet ELISA Development Systems (R&D Systems, Minneapolis, MN) and vWF was measured using Von Willebrand Factor BioAssay ELISA (USBiological Life Sciences, Salem, MA). All assays were performed according to the manufacturer's instructions. For each patient, samples collected before and after intervention were analyzed on the same ELISA plate.

2.4. Statistical analysis

We calculated that a sample size of 120 patients would give a power of 80% to detect a small effect (Cohen's $d = 0.20$) with an alpha of 0.05 and an inter-correlation of 0.70 between the two measurements. Normality of the data and homogeneity of variances were tested using the Shapiro–Wilks test and Levene's test. Variables before and after the dietary intervention were expressed as mean with standard deviation or median with inter-quartile range and tested for statistical significance using a two-sided paired samples t-test or a Wilcoxon ranking test plus Hodges–Lehman median difference test, depending on presence or absence of normality of data. Wilcoxon ranking tests were used to compare the distribution of participants across CRP based CVD-risk groups before and after the intervention. Matched linear regression analyses were performed to test the association of the change in biomarkers with medication use and T2D history, corrected for age, sex and change in insulin dose. To analyze the interaction between the biomarkers before and after dietary intervention and medication use, we used repeated measurements MANOVA analyses. The variables used in the regression analyses and repeated measurements MANOVA were Blom-transformed to meet with the assumption of normally distributed residuals. Statistical significance was considered at p-values of <0.05 . All statistical analyses were carried out using IBM SPSS statistics version 21.

3. Results

3.1. Baseline characteristics

Table 1 summarizes the characteristics of our study population. At baseline, the BMI was $36.8 \pm 5.6 \text{ kg/m}^2$. The median period after T2D diagnosis was 10.0 years and ranged from 0.4 to 39.0 years. Of the 131 participants, 62.6% were treated with insulin, 75.6% with metformin and 20.6% with a sulfonylurea-derivative, while only two participants were treated with incretins and none with thiazolidinediones. 72.5% of the participants used statins, whereas 58.8% of the participants used ACE inhibitors, 35.9% beta blockers, 21.4% calcium antagonists, 48.1% diuretics and 3.1% alpha-2 antagonists. Microvascular and macrovascular complications were evident in 60.3% and 27.6% of the participants. While on medication, the median baseline HbA1c level was 61.0 mmol/mol and the median baseline LDL cholesterol level was 2.5 mmol/l. Nineteen participants (15.4%) were current smokers; during the intervention period 3 started and 2 stopped smoking.

At baseline, the statin users had a lower median LDL cholesterol (2.4 mmol/L (2.0, 3.0) vs. 2.9 mmol/L (2.3, 3.6), $p = 0.007$), lower hs-CRP (3.3 g/mL (1.3, 10.0) vs. 6.9 g/mL (1.8, 18.9), $p = 0.035$) and lower vWF levels (30.6 IU/mL (23.1, 41.3) vs. 36.2 IU/mL (27.5, 57.7), $p = 0.028$) than the non-statin users. Compared to participants not using ACE inhibitors, users were older (56.0 y (50.0, 63.0) vs. 53.0 y (42.5, 59.0), $p = 0.018$) and had a higher systolic blood pressure (141.0 mmHg (130.0, 162.5) vs. 132.0 mmHg (122.5, 148.5), $p = 0.009$). Participants on metformin treatment did not differ at baseline from those not using metformin. Participants on 'maximum treatment' using the combination of statins, ACE inhibitors and metformin ($n = 48$) showed a lower hs-CRP level than the other participants (2.7 g/mL (1.1, 7.1) vs. 4.8 g/mL (2.3, 16.6), $p = 0.015$).

3.2. Effect of the dietary intervention

The effects of the dietary intervention on metabolic and endothelial markers are shown in **Table 1**. During the 4-month dietary

intervention, participants lost 10.2 kg (95%CI 9.2, 11.3; $p < 0.001$), which is $9.8 \pm 5.2\%$ of their initial bodyweight. The median plasma levels of HbA1c (-7.0 mmol/mol (95%CI $-8.5, -5.0$); $p < 0.001$), LDL cholesterol (-0.2 mmol/L (95%CI $-0.4, -0.1$); $p < 0.001$) and other plasma lipids ($p < 0.01$) improved, while systolic blood pressure did not change ($p = 0.405$). The median plasma levels of sICAM-1 (-22.4 ng/mL (95%CI $-37.1, -8.7$); $p = 0.001$), vWF (-3.9 IU/mL (95%CI $-6.4, -1.4$); $p = 0.003$) and hs-CRP (-0.6 mg/L (95%CI $-1.2, -0.2$); $p = 0.007$) decreased during the weight loss intervention, while sVCAM-1 levels were unchanged (1.6 ng/mL (95%CI $-41.5, 48.6$); $p = 0.949$). The change in these biomarkers was not correlated with change in physical activity nor with the prevalence of microvascular and macrovascular disease (data not shown).

Only for CRP levels, a cut-off point for high CVD risk has been defined as equal to or above 3 mg/L, while CRP levels $<1 \text{ mg/L}$ reflect a low risk for CVD [12]. The distribution of patients over the three risk categories shifted significantly towards the lower CVD-risk categories during the diet intervention ($p < 0.001$): at baseline, 58.3% of our population had a CRP level above 3 mg/ml, which decreased to 50.8% after the dietary intervention ($p = 0.033$). The proportion of patients with a low-risk CRP level increased from 12.1% to 23.5% during the diet intervention ($p < 0.001$).

3.3. Associations of the change in biomarkers with duration of T2D

To determine whether the diet-induced changes in biomarkers were associated with the time since diagnosis of T2D, we conducted univariate linear regression analyses, corrected for age, sex and change in insulin dose. At baseline, sVCAM-1 levels were positively associated with duration of T2D ($\beta = 0.245$, $p = 0.005$), while sICAM-1 ($\beta = 0.104$, $p = 0.237$), vWF ($\beta = 0.065$, $p = 0.460$) and hs-CRP levels ($\beta = -0.021$, $p = 0.811$) were not. The diet-induced changes in sICAM-1 ($\beta = -0.044$, $p = 0.621$), sVCAM-1 ($\beta = 0.013$, $p = 0.886$), vWF ($\beta = -0.005$, $p = 0.952$) and hs-CRP ($\beta = -0.012$, $p = 0.887$) were not related to the duration of T2D.

Table 1
Characteristics of the study population ($n = 131$) before and after dietary intervention.

Variables ^a	Before	After	p-value ^b
Age (y, range)	54 (26–74)		
Sex (% female)	57.3		
Years after diagnose T2D	10.0 (3.0, 15.0)		
Insulin users (%)	62.6		
Insulin dose among users (IU/day)	108.0 (68.0, 136.0)	31.0 (16.8, 52.5)	<0.001
Metformin users (%)	75.6	unchanged	
Metformin dose among users (mg/day)	1700 (1000, 2550)	unchanged	
Statin users (%)	72.5	unchanged	
ACE inhibitor users (%)	58.8	unchanged	
Microvascular disease (%)	60.3	unchanged	
Macrovascular disease (%)	27.6	unchanged	
Physical activity (SQUASH score)	3360 (1440, 6270)	3960 (1800, 7680)	0.395
Weight (kg)	105.0 \pm 19.1	94.5 \pm 17.3	<0.001
BMI (kg/m^2)	36.8 \pm 5.6	33.1 \pm 5.2	<0.001
Waist circumference (cm)	119.8 \pm 12.9	110.8 \pm 11.9	<0.001
Systolic blood pressure (mmHg)	138.0 (127.8, 157.3)	139.0 (128.0, 150.0)	0.405
HbA1c (mmol/mol)	61.0 (52.0, 71.0)	53.0 (43.0, 66.0)	<0.001
Fasting glucose (mmol/l)	8.8 (6.9, 10.8)	7.3 (6.1, 9.3)	<0.001
Total cholesterol (mmol/l)	4.4 (3.7, 5.1)	4.1 (3.5, 4.8)	<0.001
LDL cholesterol (mmol/l)	2.5 (2.1, 3.1)	2.4 (1.8, 2.9)	<0.001
HDL cholesterol (mmol/l)	1.1 (1.0, 1.3)	1.2 (1.0, 1.4)	0.002
Triglycerides (mmol/l)	1.8 (1.2, 2.6)	1.4 (1.0, 2.0)	<0.001
sICAM-1 (ng/ml)	175.0 (133.1, 257.5)	158.2 (113.4, 222.5)	0.001
sVCAM-1 (ng/ml)	451.3 (324.3, 636.5)	432.5 (292.0, 682.1)	0.949
hs-CRP (mg/l)	3.9 (1.6, 13.0)	3.4 (1.1, 8.5)	0.007
vWF (IU/ml)	31.3 (24.1, 43.6)	30.2 (20.5, 40.7)	0.003

^a Mean \pm SD or median (interquartile range).

^b Paired samples T-test or Wilcoxon ranking test.

3.4. Associations of the change in biomarkers with cardio-protective medication

Firstly, we studied the association of medication with the diet-induced change in the vascular biomarker levels via a matched linear regression analysis corrected for age, sex and change in insulin dose. Changes in the biomarkers were not associated with the use of statins, ACE inhibitors or metformin, except for a negative association between statin use and change in sVCAM-1 ($\beta = -0.23$, $p = 0.020$).

We also performed a formal interaction study. In Table 2 we present the difference in effect of the dietary intervention on the measured vascular biomarkers, according to medication use. There were no significant interactions between the vascular biomarker levels before and after diet and the use of statins, ACE inhibitors and metformin. We found a borderline significant interaction between sVCAM-1 before and after diet with maximum medical treatment (statin plus ACE inhibitor plus metformin). Participants on maximum medical treatment showed a decrease in sVCAM-1 while the other participants showed an increase in sVCAM-1 (between-group difference $p = 0.047$). Similar results were obtained after adjusting for age, sex, weight change and insulin use.

4. Discussion

We found that diet-induced weight loss not only improved glycemic state and lipid parameters, but also reduced biomarkers of endothelial dysfunction and inflammation in overweight and obese patients with T2D. These effects of weight loss were not associated with the duration of T2D or with the use of statins, ACE inhibitors, and metformin.

The beneficial effect of diet-induced weight loss on classical cardiovascular risk factors such as hypercholesterolemia and hyperglycemia in obese T2D patients is well-established [20,23] and confirmed in this study. Our results on biomarkers of endothelial function and inflammation are in concordance with previous studies on weight loss dieting in obese subjects with and without T2D [16,19,24–27]. The biomarkers for endothelial function used in

this study play an important role in the adhesion of leukocytes to the endothelium, coagulation processes and vascular inflammation [28,29]. In subjects with T2D, each of these biomarkers is strongly associated with cardiovascular outcome [3,7–11], and a lowering of sICAM-1, vWF and hs-CRP may therefore reduce the risk of vascular complications. To illustrate, the proportion of patients shifting from the medium or high CVD risk group to the low CVD risk group, according to their hs-CRP level, was almost doubled. However, the changes in biomarker levels induced by our short term diet-induced weight loss program are relatively small, and follow-up studies are required to determine the clinical relevance.

We found that statin use and diet-induced weight loss are additive in reducing hs-CRP levels in T2D patients, which is in line with the results of the Look AHEAD trial [24]. Although the dietary intervention in our study was of shorter duration, the same magnitude of weight loss was observed as in the Intensive Lifestyle Intervention arm of the Look AHEAD trial after 1 year [20]. Unlike the Look AHEAD group, we directly compared the statin-treated with the non-statin treated participants. Adjustments of the statin doses made during the dietary intervention period were a limitation of the Look Ahead study. In our study, medication doses were maintained during the intervention. We found no interaction of statin use with improvements in hs-CRP levels, nor in sICAM-1 and vWF levels. The only interaction between diet and medication was observed in patients receiving the combination of a statin, ACE inhibitor and metformin versus the other patients, which showed a diet-induced improvement of sVCAM1 levels when on maximal treatment. The lack of effect on cardiovascular endpoints in the Look AHEAD trial [20] may therefore not be the result of the increasingly intensive medical treatment of the participants, but possibly of the gradual regain of weight during the follow-up period. Taken together, lifestyle interventions aimed at long-term weight reduction may still be a therapeutic option on top of medication in overweight and obese T2D patients.

The wide range of T2D history in our study group (0.4–39.0 years since T2D was diagnosed) gave us a good opportunity to study the influence of duration of T2D on the effect of a weight loss intervention. One could argue that in a late stage of disease many

Table 2

The interaction between diet-induced weight loss and medication on markers of endothelial function and inflammation.

Biomarker:	Before diet:	After diet:	Before diet:	After diet:	P _{time} *	P _{interaction} **
No statins (n = 36)						
sICAM-1 (ng/mL)	182.9 (127.7, 281.0)	150.2 (110.6, 258.0)	174.7 (134.0, 249.1)	158.2 (116.0, 216.8)	0.002	0.711
sVCAM-1 (ng/mL)	409.0 (325.0, 588.6)	474.9 (339.2, 724.5)	459.7 (323.4, 686.0)	424.3 (276.7, 635.9)	0.667	0.079
hs-CRP (g/mL)	6.9 (1.8, 18.9)	3.8 (1.4, 11.7)	3.3 (1.3, 10.0)	2.6 (0.9, 7.5)	0.001	0.232
VWF (IU/mL)	36.2 (27.5, 57.7)	31.8 (21.9, 49.9)	30.6 (23.1, 41.3)	29.3 (18.6, 38.1)	0.012	0.657
No ACE inhibitors (n = 54)						
sICAM-1 (ng/mL)	181.1 (134.9, 258.2)	193.6 (127.0, 251.0)	171.1 (128.3, 251.6)	145.4 (107.7, 200.5)	0.003	0.053
sVCAM-1 (ng/mL)	420.6 (303.6, 579.2)	438.8 (294.8, 736.4)	476.1 (333.7, 663.6)	432.5 (274.2, 675.7)	0.854	0.220
hs-CRP (g/mL)	3.8 (1.8, 13.1)	3.5 (1.5, 8.6)	4.4 (1.3, 12.6)	2.7 (0.8, 8.6)	0.003	0.253
VWF (IU/mL)	31.9 (27.1, 39.9)	31.4 (25.0, 41.2)	31.3 (22.6, 48.4)	28.4 (17.9, 39.2)	0.018	0.247
No metformin (n = 32)						
sICAM-1 (ng/mL)	217.8 (138.0, 298.6)	204.8 (130.0, 285.6)	171.1 (127.8, 231.1)	146.8 (109.1, 202.6)	0.020	0.248
sVCAM-1 (ng/mL)	484.5 (306.4, 730.0)	441.9 (339.2, 918.8)	429.3 (327.1, 614.4)	424.4 (276.7, 680.4)	0.988	0.478
hs-CRP (g/mL)	5.4 (2.4, 17.0)	5.3 (1.7, 22.7)	3.6 (1.3, 11.6)	2.6 (0.9, 7.4)	0.047	0.094
VWF (IU/mL)	33.0 (24.5, 56.5)	32.2 (15.6, 48.1)	31.0 (24.1, 42.7)	29.9 (20.5, 39.8)	0.027	0.948
No maximum treatment (n = 83)						
sICAM-1 (ng/mL)	186.9 (133.8, 285.7)	189.8 (125.5, 262.2)	161.9 (128.6, 207.9)	142.6 (98.0, 168.1)	0.001	0.093
sVCAM-1 (ng/mL)	432.3 (317.5, 610.7)	439.1 (335.2, 736.2)	461.0 (332.6, 669.1)	420.6 (248.4, 568.9)	0.353	0.047
hs-CRP (g/mL)	4.8 (2.3, 16.6)	4.0 (1.6, 11.7)	2.7 (1.1, 7.1)	2.0 (0.7, 7.0)	0.002	0.887
VWF (IU/mL)	33.7 (26.2, 43.6)	31.3 (22.4, 42.9)	28.1 (21.5, 45.9)	28.0 (17.8, 38.1)	0.007	0.515
Maximum treatment (n = 48)						

Median (interquartile range) of the biomarkers are reported.

Repeated measurements MANOVA analyses were performed on normalized outcome values, we reported the *time effect and **interaction effect.

pathological changes have become irreversible and therefore the vasculature may not benefit from weight loss anymore [30]. However, we found that the effect of weight loss on surrogate vascular endpoints was independent of T2D duration, suggesting that patients with a long history of T2D still benefit from a weight loss intervention similar to newly diagnosed patients. Our results are in line with results from patients with a relatively short history of T2D. In the ACTID trial [26] the levels of sICAM-1, CRP and IL-6 decreased significantly during diet and in another small trial brachial flow-mediated dilatation (FMD) improved, although the microvascular reactivity and vWF did not change significantly in this latter trial [19].

4.1. Strengths and limitations of the study

The present study is a pragmatic before-after study in the run-in phase of a randomized trial. As a consequence, we cannot fully exclude that other factors than the dietary intervention have contributed to the described effects. Physical activity did not change significantly and did not correlate with the change in biomarkers (data not shown), medication use was tightly monitored and no major changes in medical status are to be expected during the 4-month intervention period. However, patient compliance to medication or dietary treatment could not be checked. Future randomized controlled replication studies are required in order to confirm our findings. One could argue that the medication subgroups were small and that the chance of a type 2 error was considerable for the interaction analysis. However, the linear regression analyses are more robust and were in concordance with the interaction study. Another limitation is the use of change scores in the regression analyses, which may be sensitive to regression toward the mean. Finally, our study was limited to the endothelial role in inflammation and thrombosis using surrogate markers. We have no data available for vasomotor, barrier and angiogenic function. The effect of diet-induced weight loss on long-term endothelial function and cardiovascular endpoints in this patient population awaits further study. Strengths of the present study were the prospective design, structured information about medication use and a strict medication protocol during the intervention. Furthermore, we were able to collect a cohort with a wide range of T2D history, making the results generalizable to the overweight and obese T2D population.

4.2. Conclusions

Our findings support a beneficial effect of diet-induced weight reduction on biomarkers of endothelial dysfunction and inflammation irrespective of intensive medical drug treatment and a long history of T2D. Replication in long term follow-up studies is needed in order to establish the effect of diet-induced weight loss and the interaction with cardio protective medication on cardiovascular endpoints.

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Statement of authorship

KAB participated in the design of the study, recruited the participants, led the intervention, participated in the statistical analyses and wrote the first draft of the manuscript. TPO participated in the design of the study, the measurement of the biomarkers and the

statistical analyses. RT analyzed the data statistically and revised the manuscript. WAD, MTM, AJMV, AJMR, and EJGS participated in the design and coordination of the study and revised the manuscript critically. EJGS supervised the research and is the guarantor of this work, having full access to the data and takes the responsibility for the integrity and accuracy of the data and the analyses. All authors read and approved the final manuscript.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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