Targeting Inflammation Using Salsalate in Patients With Type 2 Diabetes: Effects on Flow-Mediated Dilation (TINSAL-FMD)

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OBJECTIVE—To test whether inhibiting inflammation with salsalate improves endothelial function in patients with type 2 diabetes (T2D).

RESEARCH DESIGN AND METHODS—We conducted an ancillary study to the National Institutes of Health–sponsored, multicenter, randomized, double-masked, placebo-controlled trial evaluating the safety and efficacy of salsalate in targeting inflammation to improve glycemia in patients with T2D. Flow-mediated, endothelium-dependent dilation (FMD) and endothelium-independent, nitroglycerin-mediated dilation (NMD) of the brachial artery were assessed at baseline and 3 and 6 months following randomization to either salsalate 3.5 g/day or placebo. The primary end point was change in FMD at 6 months.

RESULTS—A total of 88 participants were enrolled in the study, and data after randomization were available for 75. Patients in the treatment and control groups had similar ages (56 years), BMI (33 kg/m^2), sex (64% male), ethnicity, current treatment, and baseline HbA_{1c} (7.7% [61 mmol/mol]). In patients treated with salsalate versus placebo, HbA_{1c} was reduced by 0.46% (5.0 mmol/mol; $P < 0.001$), fasting glucose by 16.1 mg/dL ($P < 0.001$), and white blood cell count by 430 cells/μL ($P < 0.02$). There was no difference in the mean change in either FMD (0.70% [95% CI −0.86 to 2.25%]; $P = 0.38$) or NMD (−0.59% [95% CI −2.70 to 1.51%]; $P = 0.57$) between the groups treated with salsalate and placebo at 6 months. Total and LDL cholesterol were 11 and 16 mg/dL higher, respectively, and urinary albumin was 2.0 μg/mg creatinine higher in the patients treated with salsalate compared with those treated with placebo (all $P < 0.009$).

CONCLUSIONS—Salsalate does not change FMD in peripheral conduit arteries in patients with T2D despite lowering HbA_{1c}. This finding suggests that salsalate does not have an effect on vascular inflammation, inflammation does not cause endothelial dysfunction in T2D, or confounding effects of salsalate mitigate favorable effects on endothelial function.

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Atherosclerosis is the principal cause of death and disability among patients with type 2 diabetes (T2D), in whom it typically occurs earlier, with greater severity, and with more diffuse distribution (1). Chronic subacute inflammation is associated with obesity, insulin resistance and T2D, and atherosclerosis (reviewed by Shoelson et al. [2] and Libby [3]). Anti-inflammatory salicylates, which inhibit the nuclear factor (NF)-κB signaling pathway, are being studied as a potential new class of drugs for the treatment of these disorders, and recent studies have shown that targeting inflammation with salsalate can improve glycemia in patients with obesity and T2D (4–7).

Diabetes exerts its proatherogenic actions in part by disturbing endothelial homeostasis. Endothelium-dependent vasodilation, a marker of atherosclerosis, is impaired in patients with atherosclerotic risk factors, including diabetes (8–11). Endothelial function in peripheral conduit arteries reflects function in the human coronary circulation (12), and impaired endothelium-dependent vasodilation predicts cardiovascular events (13,14). Flow-mediated, endothelium-dependent dilation (FMD) can be quantitated as an index of vasomotor function, and the noninvasive nature of the technique permits repeated measurements over time to evaluate the effectiveness of interventions that may affect vascular health. Thus, we sought to test the hypothesis that targeting inflammation will improve endothelial function. We evaluated FMD as an informative surrogate marker of cardiovascular health following a therapeutic trial of salsalate, a novel agent being evaluated to treat T2D.

RESEARCH DESIGN AND METHODS

Trial design

The Targeting Inflammation Using Salsalate in Type 2 Diabetes (TINSAL-T2D) stage 2 trial was conducted as a single-mask lead-in, randomized, double-masked, placebo-controlled, multicenter clinical trial. In brief, the protocol, approved by human subject institutional review boards at each institution, included 1 week of screening; a 4-week, single-masked placebo run-in; a baseline evaluation before treatment; and a 48-week treatment period, with computer-generated (1:1) randomized allocation to salsalate (3.5 g/day divided) or a placebo that appeared identical, as described in

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detail (15). Inclusion criteria included T2D, age 18 to 75 years, and HbA_{1c} levels of 7–9.5% (53–80 mmol/mol) at screening. Concomitant oral diabetes medication had to be at stable dosages for at least the previous 8 weeks. Patients who received low-dose aspirin (81–325 mg/day) also were eligible for the trial. Key exclusion criteria included use of thiazolidinediones, insulin, or glucagon-like peptide-1 receptor agonists; chronic use of nonsteroidal anti-inflammatory drugs, anticoagulants, or uricosuric agents; and impaired renal function with an estimated glomerular filtration rate <60 mL/min and/or urinary albumin >300 μg/mg creatinine. The Effect of Targeting Inflammation Using Salsalate on Flow-Mediated Dilation (TINSAL-FMD) was conducted as an ancillary study to the parent trial. Seven academic sites within the United States participated in the ancillary study. The protocol was approved by the human subject institutional review boards at each institution.

Vascular function studies were performed at baseline (visit 3, week 0) before randomization; at 3 months (visit 7, week 12); and at 6 months (visit 9, week 24). All nitric oxide potentiating drugs (e.g., phosphodiesterase type 5 inhibitors) were suspended for 1 week, and all antihypertensive and vasoactive drugs, alcohol, and caffeine were held for at least 12 h before vascular function measurements.

Central training and FMD methods

Endothelial function was determined in accordance with published guidelines (16). All sites received central training and underwent certification procedures to establish a uniform methodology. Each of the sites submitted to the core imaging laboratory sample images acquired from at least three volunteers for review to demonstrate the site's ability to acquire technically adequate images for interpretation before any research subject was enrolled at the site. All sites had ultrasound equipment capable of simultaneous B-mode and electrocardiographic monitoring, with an electrocardiographic trigger, DICOM file storage capacity, a Hokanson SC5 (or equivalent 6- × 83-cm) blood pressure cuff with a manual cuff inflator, and an automatic blood pressure monitor. Subjects were studied in a quiet, temperature-controlled, dimly lit room after resting supine for a minimum of 5 min. High-resolution B-mode ultrasonography of the brachial artery was performed using a 7.5-MHz linear array probe. The brachial artery was imaged longitudinally just proximal to the antecubital fossa with the transducer position adjusted to obtain optimal images of the near and far wall of the intima. The R wave on the electrocardiogram served as a trigger to acquire digitized image frames at end diastole. After acquiring a baseline image, a sphygmomanometric cuff placed on the upper portion of the arm was inflated to suprasystolic pressure (200 mmHg) for 5 min. When the cuff is released, reactive hyperemia causes flow to increase through the brachial artery subserving the forearm. The velocity time integral was assessed by pulse Doppler within 15 s of cuff release to assess the peak velocity of hyperemic blood flow. FMD of the brachial artery was determined with images acquired 1 min after cuff deflation. FMD at this time point is largely endothelium-dependent and mediated by nitric oxide, and it can be inhibited by administration of the nitric oxide synthase antagonist N^G-monomethyl-L-arginine (17). Ten minutes after release of the cuff, the brachial artery was re-imaged to ensure a return to basal conditions. Then, to determine nitroglycerin-mediated endothelium-independent vasodilation (NMD), subjects received sublingually 0.4 mg of nitroglycerin. The brachial artery was imaged 3 min later. Nitroglycerin was not administered if the systolic blood pressure was <100 mmHg. All images were analyzed at a central core laboratory by a single reader masked to treatment assignment. In the core laboratory, the intraobserver (operator) variability is 0 ± 0.15%. Arterial diameter during each experimental condition was measured using edge detection software (Brachial Analyzer for Research, version 5.8.9 SP-2; Medical Imaging Applications LLC, Coralville, IA).

Primary and secondary outcomes

The primary outcome of this study was the change in FMD from baseline to 6-month follow-up in the group treated with salsalate compared with the group treated with placebo. Change in NMD was an important secondary outcome.

Laboratory measures

Unless otherwise noted, laboratory measurements were performed at Quest Diagnostics (Chantilly, VA). Commercial immunoassays were used according to instructions for insulin and C-peptide (Mercodia, Upsala, Sweden), adiponectin, cystatin C, high-sensitivity C-reactive protein (CRP), tumor necrosis factor (TNF) and TNF receptors (ELISA kits from R & D Systems, Minneapolis, MN), and free fatty acids (reagents from VWR International, Radnor, PA).

Statistical analyses

We analyzed the data following the intention-to-treat principle. All patients with data at the baseline FMD measurement and up to the point of dropout (if dropout occurred) were included in the analyses.

Differences in baseline characteristics were compared between treatment groups using Student *t* test for normally distributed continuous traits and χ^2 or Fisher exact test for categorical traits. For normally distributed continuous outcomes we estimated mean differences between treatment groups using linear regression mixed models, including FMD measurements adjusted for baseline levels over the 26-week study period. We assumed an autoregressive moving average covariance structure. For continuous outcomes with nonparametric distributions we used the Wilcoxon test to compare change from baseline between the placebo and salsalate groups at week 24. All statistical tests report two-sided *P* values; $P < 0.05$ was considered significant. The Holm procedure was used to correct for multiple comparisons (18).

We calculated the sample size based on mean and standard deviations of change in flow-mediated vasodilation for patients with diabetes following placebo (10). Because vascular measurements would be made at several centers we anticipated that the variation in measurements of the mean change from baseline would be 2.5-fold higher than the reported variation of 1.2%. Accordingly, based on an anticipated improvement of 25% in flow-mediated endothelium-dependent vasodilation in the group treated with salsalate compared with the group treated with placebo at 6 months and an estimated standard deviation of the mean change from baseline of 3.0%, we estimated that a total of 72 completed participants would be necessary for this study.

RESULTS

Baseline characteristics

Baseline characteristics of TINSAL-T2D subjects participating in the TINSAL-FMD ancillary study were balanced between the salsalate and placebo groups

(Table 1). We enrolled 91 participants in the ancillary study. Of these, 88 participants completed the baseline assessment and 3 were unable to schedule baseline evaluations; 68 participants had visits at baseline, 3 months, and 6 months, 7 had visits at only baseline and 3 months, and 13 had only a baseline visit (Fig. 1). We excluded patients with only a baseline visit, leaving 75 patients for whom data after randomization were available for analysis. There was no statistical difference in the sex of the participants between the treatment groups.

Participants in the TINSAL-FMD ancillary study had lower systolic blood pressure and total cholesterol and were more likely to be treated with an insulin secretagogue, antihypertensive medication (especially an angiotensin-converting enzyme inhibitor or angiotensin receptor

Table 1—*Baseline characteristics of participants in the parent TINSAL-T2D study compared with the TINSAL-FMD ancillary study and characteristics by treatment group*

Characteristics	TINSAL-T2D study (n = 286)	TINSAL-FMD study (n = 75)	Placebo (n = 38)	Salsalate (n = 37)
Age (years)	55.8 ± 9.6	56.4 ± 10.1	57.1 ± 11.8	55.6 ± 7.9
Male sex	156 (54.6)	48 (64.0)	23 (60.5)	25 (67.6)
Race/ethnicity				
White	151 (52.8)	46 (61.3)	23 (60.5)	23 (62.2)
Black	95 (33.2)	25 (33.3)	13 (34.2)	12 (32.4)
Other	40 (14.0)	4 (5.3)*	2 (5.3)	2 (5.4)
Weight (kg)	96.2 ± 22.5	97.9 ± 23.0	94.2 ± 21.7	101.7 ± 23.9
BMI (kg/m^2)	33.3 ± 6.7	33.1 ± 6.7	32.2 ± 7.5	33.9 ± 5.9
Time since diabetes diagnosed (years)[a]	4.9 (0.1, 38.3)	4.1 (0.2, 35.0)	3.6 (0.2, 35.0)	4.4 (0.6, 23.8)
Medical history				
CAD[b]	32 (11.2)	10 (13.3)	5 (13.2)	5 (13.5)
Hypertension[c]	208 (72.7)	59 (78.7)	30 (79.0)	29 (78.4)
Dyslipidemia[d]	199 (69.6)	60 (80.0)*	30 (79.0)	30 (81.1)
Blood pressure (mmHg)				
Systolic	126.1 ± 13.4	121.9 ± 13.9*	120.1 ± 15.3	123.9 ± 12.2
Diastolic	76.5 ± 8.5	74.8 ± 8.5	74.5 ± 8.1	75.2 ± 9.0
Heart rate (bpm)	72.9 ± 10.2	72.3 ± 11.9	70.4 ± 12.6	74.1 ± 10.9
Laboratory values				
HbA_{1c} (%)[e]	7.7 ± 0.7	7.7 ± 0.8	7.6 ± 0.8	7.8 ± 0.7
Fasting glucose (mg/dL)	151 ± 38	146 ± 35	143.7 ± 34.4	148.5 ± 26.7
Cholesterol (mg/dL)	165 ± 41	155 ± 42	155.2 ± 47.7	155.2 ± 36.8
Triglycerides (mg/dL)[a]	137 (45, 605)	151 (45, 531)	132 (45, 334)	163 (63, 531)
LDL (mg/dL)	102.0 ± 33.2	96.0 ± 33.6	94.8 ± 37.8	97.2 ± 29.2
Urinary albumin (μg/mg creatinine)[a]	8 (1, 350)	7 (1, 310)	7 (1, 310)	7 (2, 100)
Medications				
Metformin	252 (88.1)	68 (90.7)	34 (89.5)	34 (91.9)
Insulin secretagogue	149 (52.1)	49 (65.3)*	22 (57.9)	27 (73.0)
DPP-4 inhibitor	43 (15.0)	7 (9.3)	3 (7.9)	4 (10.8)
Lifestyle only	13 (4.6)	3 (4.0)	1 (2.6)	2 (5.4)
Monotherapy	117 (40.9)	24 (32.0)	16 (42.1)	8 (21.6)
Dual therapy	140 (49.0)	44 (58.7)	20 (52.6)	24 (64.9)
Lipid medications				
Lipid medication[f]	180 (62.9)	55 (73.3)*	26 (68.4)	29 (78.4)
Statin	171 (59.8)	52 (69.3)*	25 (65.8)	27 (73.0)
Other	28 (9.8)	9 (12.0)	3 (7.9)	6 (16.2)
Blood pressure medications				
Antihypertensive medication[g]	185 (64.7)	56 (74.7)	28 (73.7)	28 (75.7)
ACEI/ARB	159 (55.6)	49 (65.3)*	24 (63.2)	25 (67.6)
Other antihypertensive	111 (38.8)	34 (45.3)	20 (52.6)	14 (37.8)
Low-dose aspirin[h]	116 (40.6)	32 (42.7)	15 (39.5)	17 (46.0)

Data are n (%) or mean ± SD unless otherwise indicated. *$P < 0.05$ for the comparison between participants in the parent TINSAL-T2D trial with those in the TINSAL-FMD ancillary study. [a]Median (minimum, maximum) values are reported, and the statistical difference was tested on logarithmic transformed variables. [b]CAD (coronary artery disease) is defined as history of stroke, myocardial infarction, angina, or revascularization coronary artery bypass graft/percutaneous transluminal coronary angioplasty. [c]Hypertension is defined as systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg or taking antihypertensive medication. [d]Dyslipidemia is defined as LDL >150 mg/dL or taking lipid-lowering medication. [e]To convert $\%HbA_{1c}$ = [0.09148 × IFCC (mmol/mol)] + 2.152. [f]A participant can be taking both a statin and an "other" lipid medication but is counted as being on lipid medication only once. [g]A participant can be taking both an angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) and an "other" antihypertensive agent but is counted as being on antihypertensive medication only once. [h]Low-dose aspirin is 80–325 mg/day.

blocker), and lipid-lowering therapy (especially hydroxymethylglutaryl-CoA reductase inhibitors [statins]) compared with the parent trial population (Table 1).

Effect of salsalate on endothelial function

The baseline brachial artery diameter was 3.91 ± 0.62 mm in the group treated with salsalate versus 4.11 ± 0.66 mm in the group treated with placebo ($P = 0.19$). The baseline peak hyperemic blood flow, reported as the maximal hyperemic velocity time integral (the ratio of time averaged systolic velocity [centimeters/second] divided by the time of the cardiac cycle [seconds]), measured after cuff deflation, was 62.8 cm (interquartile range 42.0) in the group treated with salsalate and 69.6 cm (interquartile range 54.0) in the group treated with placebo ($P = 0.37$ between groups). There was no significant change after treatment in either brachial artery diameter or peak hyperemic velocity integral in either the salsalate or placebo treatment groups. Baseline FMD was 6.43 ± 4.55% in the salsalate group and 5.92 ± 4.02% in the placebo group. Baseline NMD was 13.14 ± 6.09% in the salsalate group and 11.78 ± 6.57% in the placebo group. Compared with placebo, salsalate had no effect on the change in FMD at either 12 or 24 weeks of treatment ($P = 0.38$) (Fig. 2*A*). Likewise, salsalate had no effect on change in NMD ($P = 0.57$) (Fig. 2*B*).

Effect of salsalate on metabolic and cardiorenal risk factors

The effects of salsalate on metabolic and cardiorenal outcomes for the entire TINSAL-T2D cohort are reported by Goldfine et al. (15). The effects were similar in magnitude for glycemic, blood pressure, lipid, and inflammatory parameters in the TINSAL-FMD cohorts. Both HbA_{1c} and fasting glucose were lowered in the group treated with salsalate compared with placebo (Table 2). There was no difference between treatment groups for systolic and diastolic blood pressures. LDL and urinary albumin, however, increased in the group treated with salsalate compared with placebo. The anti-inflammatory properties of salsalate were evident by reductions in circulating total white blood cell (WBC) and lymphocyte counts and an increase in adiponectin. There was no difference between treatment groups in the change in high-sensitivity CRP, TNF-α, or the TNF receptors 1 or 2.

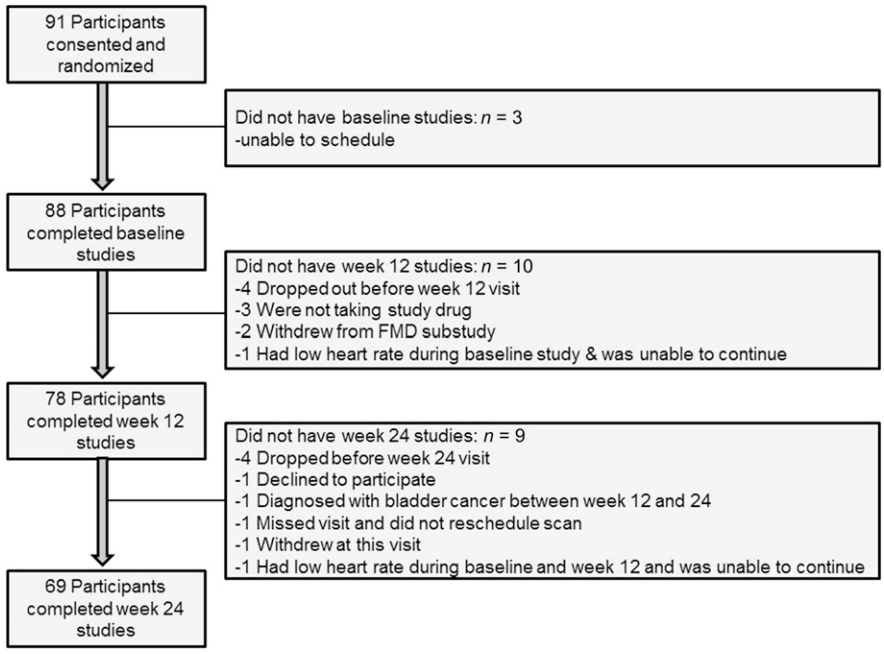

Figure 1—*TINSAL-FMD study enrollment, randomization, and retention of the study participants.*

CONCLUSIONS—The primary finding of this study is that salsalate had no effect on FMD of the brachial artery in patients with T2D. In this study we evaluated salsalate administered over 6 months at a dose that is tolerated by the majority of patients and is under evaluation for therapeutic use in the treatment of T2D. Both HbA_{1c} and fasting glucose were lowered in the group treated with salsalate compared with the group treated with placebo, demonstrating the positive

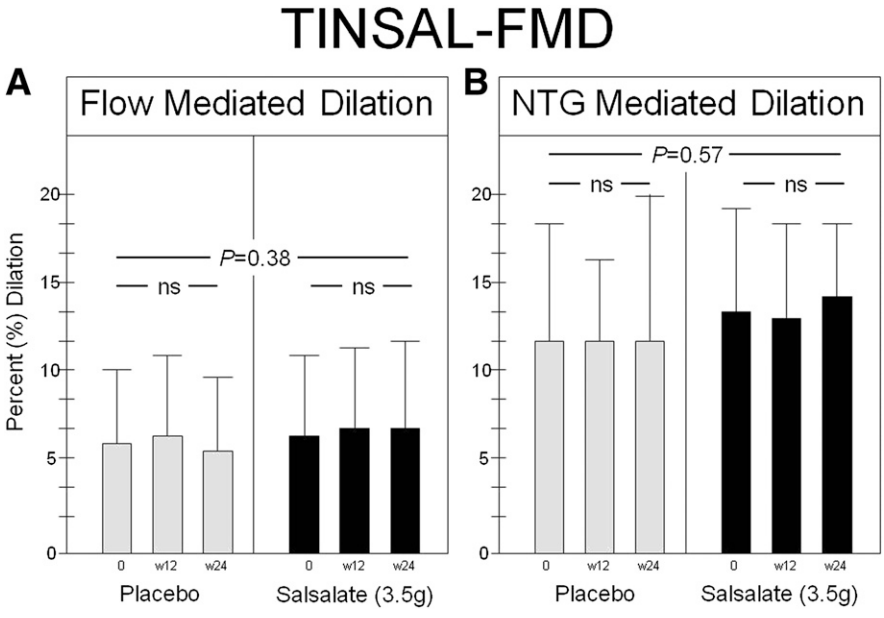

Figure 2—*FMD* (A) *and nitroglycerin (NTG)-mediated dilation* (B) *are shown for participants treated with placebo and salsalate. There was no difference in the change in FMD (the primary outcome of this study) or NTG-mediated vasodilation from baseline to the 6-month follow-up in the group treated with salsalate compared with the group treated with placebo. w12, week 12; w24, week 24.*

Table 2—*Effect of salsalate on selected clinical and laboratory values*

Outcome measure	Treatment group	Mean change (95% CI)	*P* value	Mean difference between treatment groups* (95% CI)	*P* value
Systolic blood pressure (mmHg)	Placebo	4.8 (2.6–7.1)	<0.001		
	Salsalate	4.4 (2.0–6.7)	<0.001	−0.5 (−3.7 to 2.7)	0.763
Diastolic blood pressure (mmHg)	Placebo	1.9 (0.3–3.4)	0.020		
	Salsalate	2.1 (0.5–3.7)	0.011	0.22 (−2.0 to 2.4)	0.845
HbA_{1c} (%)*	Placebo	−0.06 (−0.23 to 0.10)	0.445		
	Salsalate	−0.52 (−0.69 to −0.35)	<0.001	−0.46 (−0.69 to −0.22)	<0.001
Glucose (mg/dL)	Placebo	1.7 (−3.7 to 7.2)	0.530		
	Salsalate	−14.3 (−20.0 to −8.7)	<0.001	−16.1 (−23.9 to −8.2)	<0.001
Cholesterol (mg/dL)	Placebo	2.0 (−3.7 to 7.6)	0.495		
	Salsalate	12.8 (7.0–18.7)	<0.001	10.9 (2.8–18.9)	0.009
HDL (mg/dL)	Placebo	0.9 (−0.3 to 2.0)	0.144		
	Salsalate	1.8 (0.6–3.0)	0.004	0.9 (−0.8 to 2.6)	0.282
LDL (mg/dL)	Placebo	−1.1 (−6.2 to 4.1)	0.687		
	Salsalate	14.6 (9.2–19.9)	<0.001	15.6 (8.2–23.0)	<0.001
Triglyceride (mg/dL)†	Placebo	3.0 (−6.8 to 13.6)	0.538		
	Salsalate	−15.0 (−25.2 to −3.2)	0.014	−15.0 (−26.9 to −1.7)	0.027
Albumin-to-creatinine ratio (μg protein/mg creatinine)†	Placebo	3.4 (0.81–6.4)	0.007		
	Salsalate	25.3 (18.2–33.8)	<0.001	11.7 (6.3–4.6)	<0.001
WBC (mcL)	Placebo	20 (−22 to 26)	0.862		
	Salsalate	−41 (−67 to −16)	0.002	−43 (−79 to −8)	0.016
Lymphocytes (mcL)	Placebo	23 (−90 to 137)	0.684		
	Salsalate	−340 (−458 to −222)	<0.001	−363 (−527 to −200)	<0.001
Monocytes (mcL)	Placebo	37 (8–66)	0.013		
	Salsalate	33 (3–64)	0.033	−4 (−46 to 38)	0.858
Neutrophils (mcL)	Placebo	−66 (−289 to 157)	0.557		
	Salsalate	−113 (−348 to 121)	0.338	−47 (−372 to 277)	0.772
Free fatty acids (μmol/L)	Placebo	15.9 (−36.8 to 68.5)	0.548		
	Salsalate	33.8 (−21.5 to 89.1)	0.226	17.9 (−58.3 to 94.1)	0.640
Adiponectin (μg/mL)†	Placebo	1.1 (1.8–2.0)	0.010		
	Salsalate	6.1 (4.5–7.7)	<0.001	3.1 (1.6–4.7)	<0.001
hs-CRP (mg/L)†	Placebo	0.97 (−1.38 to 3.84)	0.573		
	Salsalate	−0.15 (−3.18 to 3.59)	0.921	−0.10 (−3.71 to 2.64)	0.643
Insulin (μU/mL)†	Placebo	−0.33 (−1.56 to 1.05)	0.717		
	Salsalate	2.06 (−0.11 to 4.19)	0.053	1.78 (−0.28 to 4.21)	0.100
C-peptide (ng/mL)	Placebo	−1.6 (−4.2 to 1.0)	0.221		
	Salsalate	−4.7 (−7.3 to −2.0)	0.001	−3.0 (−6.8 to 0.7)	0.110
TNF-α (pg/mL)†	Placebo	33.8 (29.5–38.5)	<0.001		
	Salsalate	51.0 (44.6–58.2)	<0.001	0.9 (−0.8 to 2.8)	0.309
TNFR1 (ng/mL)†	Placebo	20.3 (18.4–22.4)	<0.001		
	Salsalate	27.3 (24.6–30.1)	<0.001	−0.2 (−1.1 to 0.9)	0.742
TNFR2 (ng/mL)†	Placebo	67.1 (61.2–73.4)	<0.001		
	Salsalate	99.1 (90.3–108.7)	<0.001	0.8 (−0.5 to 2.2)	0.257

*To convert $\%HbA_{1c}$ = [0.09148 × IFCC (mmol/mol)] + 2.152. †Test is based on natural log transformation; results are back-transformed and the change ratio is multiplied by the group mean. hs-CRP, high-sensitivity CRP; TNFR, TNF receptor.

effects of salsalate on glycemia. The anti-inflammatory effects of salsalate also were evident in the lower circulating total WBC and lymphocyte counts in the group treated with salsalate, even though there was no difference in the change in CRP between groups. Potentially adverse effects on lipids and renal function were seen. Salsalate treatment increased total and LDL cholesterol but did not affect HDL; it also increased urinary albumin, albeit without changing estimates of the glomerular filtration rate.

There are several potential explanations for our findings that neither support nor refute the findings that NF-κB expression and/or activity are increased in the endothelium of people who are resistant to insulin, with differential effects in patients with and without diabetes (19,20). First, salicylate levels achieved at the tolerable doses of salsalate used in this study may have minimal or no effect on endothelial function in people with established T2D. In a report by Pierce and colleagues (21), salsalate administered to overweight patients at higher doses (4.5 g/day) over 4 days increased the inhibition of NF-κB expression, reduced nuclear expression of NF-κB in endothelial cells, and improved

endothelial function. However, these higher doses of salsalate are poorly tolerated and would not be feasible for extended administration (5,21). In addition, the positive effect of salsalate on endothelial function that was demonstrated following short-term exposure (21) may not be sustained.

Second, the participants in the TINSAL-FMD ancillary study had a higher disease index compared with the participants in the parent TINSAL-T2D study; this was manifest in the greater use of dual therapy and sulfonylureas at baseline to manage diabetes and statins and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers to manage lipids and hypertension, respectively. It is possible that targeting inflammation with salsalate has few additional beneficial effects on endothelial function in advanced disease or when added to lipid-lowering and antihypertensive therapies (22–24). Indeed, we recently found that salsalate did not change endothelial function, as assessed by peripheral arterial tonography, in patients with prediabetes, many of whom also were taking therapies to lower lipids and blood pressure (25). Third, confounding effects of salsalate on lipids and renal function may mitigate any improvements in endothelial function derived from salsalate such that the net effect is neutral. The mechanisms of increased LDL cholesterol and urinary albumin remain unknown. It is interesting that increased LDL concentrations have been observed with several classes of anti-inflammatory agents, including inhibition of TNF-α and interleukin (IL)-6 (26,27). High circulating WBC counts occur in patients with obesity and the metabolic syndrome (28) and predict incident T2D (29,30) and cardiovascular disease as well as poor outcomes in the latter (31–33), suggesting that reductions in the WBC count may benefit individuals with cardiometabolic risk. WBC counts are lowered by salsalate but, interestingly, not by statins. Salsalate lowered WBC counts in this study, even though the vast majority of study patients were taking statins. In contrast, salsalate had little effect on CRP levels in either the overall TINSAL-T2D trial or this FMD ancillary study, although CRP is lowered by statins. These findings are consistent with the notion that the anti-inflammatory effects of salsalate are independent from those of statins. Likewise, nonsteroidal anti-inflammatory drugs are not known to lower WBC counts, thus further distinguishing these anti-inflammatory drug classes and potential mechanisms.

Diets rich in calories, animal fats, and sugars have been shown to induce a state of chronic subacute inflammation and promote dyslipidemia, fatty liver, insulin resistance, T2D, and cardiovascular disease. At the molecular level, activation of the transcription factor NF-κB leads to the production of multiple mediators of inflammation. Stimuli that can activate NF-κB in obesity can be separated into extracellular ligands, such as the proinflammatory cytokines TNF-α, IL-1β, and IL-6 or fatty acids binding to Toll-like receptors, and intracellular stimuli, such as endoplasmic reticulum or oxidative stress and ceramides (2). These processes are associated with both T2D and increased atherosclerotic risk and thus could provide novel therapeutic targets to treat or prevent either or both processes. However, the overlap in inflammatory mechanisms driving diabetes and cardiovascular disease remains incompletely understood. The effect of NF-κB on the vasculature may occur through regulation of adipokines and cytokines that lead to vascular injury or by reducing insulin sensitivity within the endothelium, which normally signals the phosphatidylinositol-3 kinase and Akt/protein kinase B pathway, leading to activation of endothelial nitric oxide synthase.

Multiple studies demonstrate that high-dose salicylates inhibit activity of NF-κB (34–36), which regulates transcription of numerous inflammatory mediators. Compared with aspirin, salsalate, a nonacetylated dimer of salicylate, is an equipotent inhibitor of NF-κB but a much weaker inhibitor of the cyclooxygenase enzymes; therefore it is not associated with an increased risk of bleeding and is clinically safer. While salicylates inhibit NF-κB signaling, they may have additional cellular and molecular mechanisms of action. These include inhibition of mitochondrial dehydrogenases; transcription factors other than NF-κB (e.g., cAMP-responsive element–binding protein, NF of activated T cells, heat shock transcription factor-1); and cellular kinases (inhibitor of κB kinase-β, S6-kinase, p38 mitogen-activate protein kinase, ribosomal protein S6 kinase 2, Jun NH_2-terminal kinase) (37–41). Salicylate also may inhibit 11-β-hydroxysteroid dehydrogenase type 1 in adipose tissue (42) and activate AMP-activated protein kinase (43). It is difficult to distinguish the relative in vivo contributions of these potential mechanisms.

Finally, it is difficult to determine whether salsalate should be further developed as a diabetes therapy given the mixed effects on metabolic markers of vascular health—including glycemic improvement, increased adiponectin, and lowered WBC, triglyceride, and uric acid levels—in the setting of increased LDL cholesterol and urinary albumin. Additional studies are underway to target inflammation using salsalate in patients with diverse diseases, including schizophrenia, anemia, myelodysplastic syndromes, spinal cord injury, polycystic ovarian syndrome, and chronic obstructive pulmonary disease. Understanding the cardiovascular impact of medications that would be intended for chronic use is of high clinical importance, but the neutral effect on endothelium-dependent dilation is especially important given the many potential diseases that might improve with targeted inflammatory response.

In conclusion, in this study we targeted inflammation in patients with T2D using a clinically tolerable dose of salsalate over 6 months to evaluate the effect on atherosclerotic risk as assessed by a measure of endothelial function. We did not find a change, either improvement or worsening, in FMD of the brachial artery, a surrogate measure of nitric oxide bioactivity, despite lowering HbA_{1c} and markers of inflammation. These observations suggest that either inflammation does not cause endothelial dysfunction in T2D, that salsalate does not adequately inhibit inflammation of the vasculature, or that confounding effects of salsalate mitigate favorable effects on endothelial function. Yet while there was no measurable net effect on endothelial function, anti-inflammatory interventions potentially have other favorable effects on cardiovascular outcomes by interfering with the atherosclerotic process elsewhere. At this time, however, the net effects of salsalate on vascular health, as assessed by FMD, seem to be neutral, and further study is warranted before widespread use.

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A.B.G. and M.A.C. designed and wrote the trial protocol and wrote the manuscript. J.S.B. assisted in the development of trial materials and site training to ensure similar methods across sites and in image reading. C.D., V.F., S.E.S., and K.A.J. contributed to the manuscript. Y.-D.I.C. managed the special assay core. K.A.J. analyzed the trial data. All authors approved the final manuscript for submission. M.A.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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APPENDIX

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