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Appendix 1: Contributors

The trial protocol was designed and written by the steering committee: Steven E. Shoelson, MD, PhD (*Chair*); Allison B. Goldfine, MD; Vivian Fonseca, MD; Kathleen Jablonski, PhD; and Myrlene Staten, MD. The local institutional review boards from each participating center approved the protocol. The study statisticians, Kathleen Jablonski, PhD, and Laura Pyle, MS, analyzed the trial data. The manuscript was written by Drs. Goldfine and Shoelson, with contributions by Drs. Fonseca, Jablonski, and Staten and Ms. Pyle. The final submission was approved by Drs. Goldfine, Fonseca, Jablonski, Staten, and Shoelson and Ms. Pyle.

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Appendix 2: Methods

Trial Design

The single-blind placebo run-in period provided an interval for metabolic stabilization to assess adherence to study drugs. The study statistician produced computer-generated random-sequence assignments in a 1:1 ratio using the urn method of randomization, producing separate sequences for each clinical center in blocks of 4. All study personnel, except a limited subset at the data coordinating center, were blinded to assignment. Equal numbers were assigned to receive either salsalate or placebo (both provided by Caraco Pharmaceutical Laboratories). Patients with 80% adherence or more (assessed by pill count) to blinded placebo during the run-in phase were eligible for randomization, which was conducted in clinic blocks by using central computer assignments.

Participants and their personal physicians were asked not to change dosages of diabetes, lipid-lowering, and blood pressure medications for the first 24 weeks, if possible, to assess study drug effects. Adjustments afterward were based on good clinical practice. Adverse events were systematically assessed by questionnaires administered at each follow-up visit. Patients were instructed to monitor daily fasting glucose levels and symptomatic events using provided glucometers (LifeScan). Concurrent diabetes therapies were reduced for patients experiencing hypoglycemia, either documented by home glucose monitoring or with recurrent consistent symptoms; concurrent oral therapies were increased for documented hyperglycemia at the discretion of the primary care provider. Dosages of the study drug were reduced to the maximum tolerable dose for new or worsening tinnitus. Quality of life was assessed using the total scale and 9 subscales of the Short Form-36 survey, which reflect aspects of physical and mental health and well-being.

Criteria for terminating treatment included patient decision to withdraw consent; pregnancy or lactation; a new diagnosis of an exclusionary medical condition; an intolerable adverse event, as judged by investigator and patient; and hospitalization or surgical procedures that were probably related to the use of the study drug.

Protocol Modifications After Trial Initiation

The following 4 modifications were made to the protocol after trial initiation: The inclusion criteria were amended to improve enrollment rates by permitting use of up to 3 rather than 2 oral diabetes medications; rescue therapy for hyperglycemia was modified to permit addition of oral medications (or insulin) as they were approved by the U.S. Food and Drug Administration; the definition and management of hypoglycemia were clarified; the role of the site investigator was revised to manage diabetes concomitant medications for patient safety; and a visit after dosing was added at study week 56 to assess safety specifically for participants having new-onset tinnitus persistent at week 50, ACR greater than 30 μg/mg at week 48, an increase in systolic or diastolic blood pressure greater than 10 mm Hg at weeks 48 and 50 compared with baseline, or blood pressure greater than 150/90 mm Hg despite treatment.

Study Population

Eligible adult patients were 75 years or younger; received their diagnosis of T2DM at least 8 weeks earlier; had fasting plasma glucose concentrations of 12.5 mmol/L or less ($\leq$225 mg/dL) and HbA_{1c} levels of 7% to 9.5% at screening; and were treated with diet and exercise alone or with metformin, insulin secretagogue, or dipeptidyl peptidase-4 inhibitor, either as monotherapy or in combination. Concomitant diabetes medications were at stable dosages for more than 8 weeks. Patients receiving low-dose aspirin (81 to 325 mg/d) were eligible and were encouraged to continue use as prescribed.

Exclusion criteria included treatment with insulin, thiazolidinedione (for potential overlap in mechanism), or exenatide (associated with weight loss); intentional weight loss of 4.5 kg or more in the previous 6 months; receipt of weight-loss drugs or corticosteroids in the previous 3 months; or long-term or continuous use (daily for more than 7 days) of NSAIDs within the preceding 2 months other than low-dose aspirin (81 to 325 mg/d). We also excluded patients receiving uricosuric agents or anticoagulants other than low-dose aspirin; those with aspirin allergies; or patients having severe diabetic neuropathy, peptic ulcer disease, gastritis, unstable cardiovascular disease, uncontrolled hypertension, anemia, thrombocytopenia, hypertriglyceridemia, stage 3 or greater chronic kidney disease or proteinuria, hepatic dysfunction, preexisting chronic tinnitus, or other conditions likely to interfere with the conduct of the trial.

Adjunct Care

Medical management of the patient was the responsibility of the participant's primary care physician. However, hyperglycemic safety alerts were sent to the study team for HbA_{1c} levels greater than 10.5% during the first 24 weeks and greater than 9.5% during the latter half of the trial; TINSAL-T2D investigators were to make recommendations to the primary care physician about dosing of diabetes and concomitant medications, particularly if the participant met criteria for initiation of rescue therapy for hyperglycemia or for either severe or recurrent mild hypoglycemia. Due to the number of mild hypoglycemic events, the role of the site investigator was revised to manage diabetes concomitant medications for patient safety (as previously described).

Participants with signs and symptoms of hyperglycemia (excessive thirst, urination, or weight loss) or 3 home glucose levels greater than 13.9 mmol/L (>250 mg/dL) in 1 week were instructed to call their investigator. An interim appointment was scheduled within 1 week for additional history, examination, and fasting laboratory assessment. Confirmation of fasting glucose levels greater than 13.9 mmol/L (>250 mg/dL) warranted medication adjustments. For participants without symptoms of hyperglycemia and fasting home glucose monitoring levels greater than 13.9 mmol/L (>250 mg/dL) but for whom the fasting glucose levels on scheduled visit were greater than 13.9 mmol/L (>250 mg/dL) or HbA_{1c} levels of 10.5% or greater during the first 24 weeks of the trial or 9.5% or greater thereafter, the laboratory profile was to be repeated within 2 weeks. If similar hyperglycemia was detected on repeated evaluation, then medication adjustment was warranted.

If medication adjustments were warranted, the study investigators recommended this to the participant's primary care physician. For participants not receiving maximal metformin and sulfonylurea, treatments were maximized as follows: For persons receiving lifestyle or sulfonylurea therapy, metformin was to be added. For persons receiving submaximal metformin, metformin dosing was titrated. For persons already receiving maximal-dose metformin, glipizide was to be added. If or when metformin and sulfonylurea combination therapy was maximal and hyperglycemia adjustment was warranted, addition of a third agent was recommended (either another oral insulin or neutral protamine Hagedorn insulin [10 IU subcutaneously every evening] at the discretion of the physician). If 3 oral agents were maximized, then insulin was to be added and titrated to current practice medical goals by the investigator or clinician. Investigators and providers were cautioned that salsalate has not been specifically studied in combination with insulin. In view of the Action to Control Cardiovascular Risk in Diabetes trial results and lack of data on interaction of the study drug with insulin, we did not recommend aggressive titration of insulin. Participants were to be followed through the end of the trial, and all medication adjustments were noted.

Patients with long-term NSAID use (daily for >7 days within the preceding 2 months, other than low-dose aspirin at 81 to 325 mg daily) were excluded from the study. We recommended against use during the trial. No participants withdrew from the trial after randomization for new-onset, long-term NSAID use.

Prespecified Outcomes

The primary outcome for the TINSAL-T2D study was change in HbA_{1c} level from baseline to week 48 in the intention-to-treat population. Important secondary prespecified outcomes included change from baseline to either 48 weeks or last HbA_{1c} measurement before rescue therapy; trends in HbA_{1c} levels over time; change from baseline and trends in fasting glucose levels over time; response rates for decrease in fasting glucose levels of 1.11 mmol/L or greater ($\geq$20 mg/dL), a decrease in HbA_{1c} levels of 0.5% or greater, and a decrease in HbA_{1c} levels of 0.8% or greater; change in lipid levels (LDL cholesterol, non–HDL cho-

lesterol, triglycerides, total cholesterol, HDL cholesterol, total–HDL cholesterol ratio, and LDL–HDL cholesterol ratio); change in insulin sensitivity (insulin, C-peptide, and homeostasis model index); response rates for exceeding hyperglycemic targets between salsalate and placebo groups; need for rescue therapy; need for discontinuation of study medication; response rates in patients initially treated with lifestyle modification, insulin secretagogue, metformin, or combination therapy; response rates for a reduction in HbA_{1c} levels for obese versus nonobese participants; response rates by baseline high-sensitivity C-reactive protein level; safety and tolerability of salsalate compared with placebo; change in body weight; changes in leukocyte and differential counts, high-sensitivity C-reactive protein levels, other inflammatory markers (interleukin-6, interleukin-1β, tumor necrosis factor-α, plasminogen activator inhibitor-1, adiponectin, serum amyloid A, intercellular adhesion molecule, and vascular cell adhesion molecule), lipoproteins (apolipoproteins A and B), and free fatty acids; and change in liver function (alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase), stratified according to baseline liver function, as an index of nonalcoholic steatohepatitis and to assess potential improvements or decline. Outcomes were assessed after the final patient had completed all dosing visits. Lipoproteins and several inflammatory markers have not been analyzed to date, including interleukin-6, interleukin-1β, plasminogen activator inhibitor-1, serum amyloid A, intercellular adhesion molecule, and vascular cell adhesion molecule.

Laboratory Measurements and Calculations

Unless otherwise noted, laboratory measurements were done at Quest Diagnostics. Commercial immunoassays were used according to assay instruction for insulin and C-peptide (Mercodia), adiponectin, cystatin C, high-sensitivity C-reactive protein, and tumor necrosis factor-α (enzyme-linked immunosorbent assay [ELISA] kits from R&D Systems, Minneapolis, Minnesota), and free fatty acids (reagents from VWR International, Philadelphia, Pennsylvania).

The Mercodia Insulin ELISA has low cross-reactivity to C-peptide (<0.001) and total proinsulin (<0.01%), des-31,32 proinsulin (<0.5%), or split des-32,33 proinsulin (<0.05%), but cross-reacts with des-64,65 proinsulin (98%) and split des-65,66 proinsulin (56%), according to manufacture performance characteristics (54). The Mercodia C-peptide ELISA has low cross-reactivity to intact insulin (<0.001%), with the following cross-reactivity to total proinsulin: <1.8%; des-31,32 proinsulin: 3%; or split des-32,33 proinsulin: 2%, des-64,65 proinsulin: 74%, and split des-65,66 proinsulin: 10%, according to manufacture performance characteristics (54).

To estimate the GFR, the Modification of Diet in Renal Disease formula was used: estimated GFR = $186 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times (1.212 \text{ if black}) \times (0.742 \text{ if female})$; with creatinine in mg/dL, and age in years. Creatinine levels in μmol/L can be converted to mg/dL by dividing them by 88.4. Serum cystatin C GFR was calculated as the reciprocal of cystatin C (mg/L) multiplied by 86.7 and reduced by subtracting 4.2, as described (2).

Missing Data for the Primary Analysis

Three participants randomly assigned to placebo are missing all data on HbA_{1c} levels. Two withdrew consent from the trial immediately after randomization and before a blood draw, and 1 withdrew consent to have any blood draws, stopped study medication, attended through week 24, then withdrew all consent to participate. Therefore, we do not have results to analyze for these patients.

Secondary Outcomes

The cumulative changes in concomitant diabetes medications by treatment group are shown in **Appendix Table 1**. Concomitant diabetes medications used by participants at the end of the study by treatment group are shown in **Appendix Table 2**. The number of patients reporting dyspepsia or nausea and vomiting were equal between groups (**Appendix Table 3**).

In mixed-model analyses, HbA_{1c} response rates did not differ in patients with baseline obesity (body mass index $\geq$30 kg/m^2) ($P = 0.725$) or elevated high-sensitivity C-reactive protein levels greater than 285 nmol/L (>3 mg/L) ($P = 0.62$).

In separate exploratory mixed-model analyses to assess the relationship between the change in inflammatory marker or mediators, we found the change in adiponectin inversely correlated with change in both HbA_{1c} levels (β estimate, -0.043 [CI, -0.071 to -0.015]; $P = 0.001$) and fasting glucose levels (β estimate, -2.00 [CI, -3.74 to -0.26]; $P = 0.023$) in the salsalate group but not in the placebo group ($P = 0.88$ for HbA_{1c}; $P = 0.83$ for glucose). Although change in high-sensitivity C-reactive protein levels did not differ between groups (**Table 2**), in the separate exploratory mixed-model analyses there were also statistically significant associations between change in high-sensitivity C-reactive protein and either change in HbA_{1c} levels (β estimate, 0.01 [CI, 0.00 to 0.02]; $P = 0.037$) or fasting glucose levels (β estimate, 0.75 [CI, 0.14 to 1.36]; $P = 0.016$), in the salsalate but not the placebo group ($P = 0.68$ for HbA_{1c}; $P = 0.37$ for glucose).

There were 76 (50%) aspirin users in the salsalate group and 64 (48%) in the placebo group ($P = 0.79$, chi-square). In a mixed-model analysis, there was no interaction between salsalate or placebo and baseline aspirin use ($P = 0.61$).

Plausible reasons why statin use may confound and attenuate the glycemic effect of salsalate include the established anti-inflammatory properties of statins and the association between statins and new-onset diabetes. There was no interaction between group and statin use at baseline in predicting change in fasting glucose ($P = 0.52$) or HbA_{1c} levels ($P = 0.75$). However, in an exploratory analysis using a mixed-model analysis adjusted for group, statin use was an independent predictor of the change in fasting glucose levels ($P = 0.025$), with a trend ($P = 0.072$) toward greater fasting glucose level decreasing in participants randomly assigned to salsalate receiving statins at baseline (-0.98 mmol/L [-17.66 mg/dL] [CI, -1.26 to -0.68 mmol/L {-22.70 to -12.25 mg/dL}]; $P < 0.001$) compared with those not receiving statins at baseline (-0.46 mmol/L [-8.29 mg/dL] [CI, -0.84 to -0.08 mmol/L {-15.14 to -1.44 mg/dL}]; $P = 0.018$). In contrast, the difference in change in fasting glucose

levels was not significant ($P = 0.34$) for patients randomly assigned to placebo who were receiving statins at baseline (0.10 mmol/L [1.80 mg/dL] [CI, −0.15 to 0.35 mmol/L {−2.70 to 6.31 mg/dL}]; $P = 0.44$) compared with those not receiving statins at baseline (0.29 mmol/L [5.23 mg/dL] [CI, −0.02 to 0.60 mmol/L {−0.36 to 10.81 mg/dL}]; $P = 0.066$). Likewise, greater glycemic-decreasing trends ($P = 0.144$) were numerically similar for change in HbA_{1c} levels for salsalate recipients who were receiving statins at baseline (−0.42% [CI, −0.57% to −0.27%]; $P < 0.001$) compared with those not receiving statins at baseline (−0.21% [CI, −0.40% to −0.02%]; $P = 0.030$). In contrast, the difference in change in HbA_{1c} levels was not significant ($P = 0.58$) for placebo recipients who were receiving statins at baseline (0.03% [CI, −0.01 to 0.16%]; $P = 0.65$) compared with those not receiving statins at baseline (0.08% [CI, −0.07 to 0.24%]; $P = 0.28$). Taken together, these data suggest that the glycemic efficacy of salsalate is greater, not attenuated, in statin users. Differences in statistical significance for interactions between salsalate and statin use in fasting glucose versus HbA_{1c} levels may be due to different time intervals between glycemic assessment captured by fasting glucose and HbA_{1c} levels, contributions of nonfasting glycemia to HbA_{1c} levels, or a type I statistical error. In view of the negative statistical interaction between statins and salsalate, these findings are provocative and interesting but inconclusive.

The interaction between statin use at baseline and treatment group was not statistically significant for the lipid outcomes fasting total cholesterol ($P = 0.124$), HDL cholesterol ($P = 0.57$), LDL_{direct} ($P = 0.106$), or triglyceride levels (log-transformed, $P = 0.93$).

We saw no statistically significant difference in the change in alanine aminotransferase, aspartate aminotransferase, or γ-glutamyltransferase in patients with elevated levels at baseline between the salsalate and placebo groups, using Kruskal–Wallis testing followed by Wilcoxon rank-sum test pairwise comparisons.

54. **Mercodia.** Mercodia Insulin ELISA. Uppsala, Sweden: Mercodia. Accessed at www.mercodia.se/products/human.html on 9 May 2013.

Appendix Table 1. **Time to First Adjustment of Concomitant Diabetes Medication***

Medication	8 wk	12 wk	16 wk	24 wk	36 wk	48 wk
Placebo						
Increase	6	7	8	21	28	32
Decrease	0	2	2	2	4	5
Salsalate						
Increase	3	4	5	7	14	15
Decrease	14	16	18	19	21	24

* Cumulative adjustments in concomitant diabetes medications showing number of patients by treatment group. The first adjustment per participant was included, with increases or decreases shown separately.

Appendix Table 2. **Use of Concomitant Diabetes Medications at End of Study**

Medication	Total, *n/N (%)*	Placebo, *n/N (%)*	Salsalate, *n/N (%)*
Metformin	204/238 (85.7)	104/116 (89.7)	100/122 (82.0)
Insulin secretagogue	115/238 (48.3)	51/116 (44.0)	64/122 (52.5)
Insulin	14/238 (5.9)	9/116 (7.8)	5/122 (4.1)
α-Glucosidase inhibitor	1/238 (0.4)	0/116 (0)	1/122 (0.8)
DPP-4 inhibitor	32/238 (13.4)	16/116 (13.8)	16/122 (13.1)

DPP-4 = dipeptidyl peptidase-4.

Appendix Table 3. **Adverse Events Occurring in ≥5% of the Salsalate Group and More Frequently in the Salsalate Group Than in the Placebo Group**

Condition	Total, *n/N*	Placebo, *n/N*	Salsalate, *n/N*	*P* Value*
Tinnitus	23/286	7/140	16/146	0.082
Frequent cough	39/286	19/140	20/146	1.00
Vomiting	21/286	10/140	11/146	1.00
Muscle stiffness	23/286	9/140	14/146	0.39
Dizzy	21/286	10/140	11/146	1.00
Weakness or fatigue	22/286	9/140	13/146	0.51

* Fisher exact test.

Appendix Table 4. **Incidence of Gastrointestinal Side Effects, by Treatment Group**

Condition	Placebo, *n/N*	Salsalate, *n/N*
Heartburn	15/140	15/146
Trouble swallowing	1/140	1/146
Nausea	12/140	8/146
Vomiting	10/140	11/146