Protocol Amendment: Version 2.7

Modifications of the protocol for performance of the end of study MDCTA for study subjects with heart rate above 65bpm upon arrival at BIDMC’s Department of Radiology. These changes are a revision of the then current protocol used by the radiology department which were approved in version 2.3. Previously, the dosing criteria was based solely on the heart rate and did not take the blood pressure into consideration. The new criteria for metoprolol dosing are:

Version 2.7:

- For patients who did NOT take their β-blocker, give them their usual daily dose 1 hour before scan.

- For all patients regardless of heart rate:
  - SBP < 100 - no metoprolol
  - SBP 100 – 105 - should receive a maximum 25mg of metoprolol
  - SBP 106 - 110 - should receive a maximum of 50mg of metoprolol

- For patients with SBP > 110 and who do not normally take a β-blocker:
  - For heart rates > 81 - maximum 100mg metoprolol
  - For heart rates 76-80 - maximum 75mg metoprolol
  - For heart rates 71-75 - maximum 50mg metoprolol
  - For heart rates 65-70  - maximum 25mg metoprolol

- If necessary, IV Metoprolol can be given in 5 mg doses every 5 minutes to 20 mg total.

- For patients who took their β-blocker more than 1 hour prior to initial vitals being taken:
  - For heart rates > 81 – give a maximum additional 75mg metoprolol
  - For heart rates 65-80 - give a maximum additional 50mg metoprolol

Version 2.3
For subjects with a heart rate greater than this, the beta-blocker Metoprolol (50 mg) will be administered by mouth to reduce the rate to less than 65 beats per minute one hour before the MDCTA. If the subject’s heart rate remains greater than 65 beats per minute at 1 hour after 50 mg of metoprolol is given by mouth, additional metoprolol will be given per the current radiology protocol until the heart rate goes down to 65 beats per minute.

Protocol Amendment: Version 2.6

Modification of the protocol for performance of end of study MDCTA for study subjects with creatinine up to 1.9 mg/dL. The clinical radiology protocol for the Beth Israel Deaconess Medical Center will be utilized.

- For Cr level of 1.3 to 1.6 mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of Optiray 320 or 350.

- For Cr level of 1.7 to 1.9 mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of 100cc of Visipaque.
Two weeks before end of treatment visit, subjects will have blood drawn to check BUN, Cr and eCrCLCG. If eCrCLCG is <55 ml/min or serum Cr > 1.3 mg/dL, subjects will drink additional water for 3 days and have blood work repeated. If eCrCLCG continues to be <55 ml/min or serum Cr > 1.3 mg/dL, subjects will not undergo the final MDCTA but will finish all other aspects of the study, we will use the Beth Israel Deaconess Hospital clinical radiology protocol for hydration: Cr level 1.3 to 1.6 mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of Optiray 320 or 350, and for Cr level of 1.7 to 1.9 mg/dL: oral hydration pre and post administration of 100cc of Visipaque. At the end of the visit at month 30, subjects will be dismissed from the study and returned to the care of their primary and cardiac care physicians.

Version 2.4
Two weeks before end of treatment visit, subjects will have blood drawn to check BUN, Cr and eCrCLCG. If eCrCLCG is <55, subjects will drink additional water for 3 days and have blood work repeated. If eCrCLCG continues to be <55, subjects will not undergo the final MDCTA but will finish all other aspects of the study.

Section 5.2.4, page 66 and Section 6.2.1.4, page 73
For those few subjects who may have a Cr >1.3 mg/dL or CrCl < 55 ml/min, we will use the Beth Israel Deaconess Hospital clinical radiology protocol for hydration: Cr level 1.3 to 1.6 mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of Optiray 320 or 350. For Cr level of 1.7 to 1.9 mg/dL: oral hydration pre and post administration of 100cc of Visipaque.

Version 2.4
Those with eCrCLCG < 55 ml/min at 30 month follow-up are excluded from MDCTA.

Protocol Amendment 2.5

Section 3.6 Statistical Analysis, pg. 61
The following statement has been added regarding the analysis of data for early termination subjects.

We will assess the effects of missing data in the final dataset using a sensitivity analysis, imputing missing values using various optimistic and pessimistic assumptions (no change, mean/median change in the active treatment arm, mean/median change in the standard care arm, etc.) to assess the magnitude of any biased losses. Data from early assessment of the primary outcome in those participants who drop out of the trial will be used to determine the validity of these assumptions. We will also perform multiple imputation analysis using standard algorithms. Because missing data may represent informative censoring, we will also perform a formal survival analysis of both the intervention and placebo groups to determine if there has been any biased loss to follow-up.

Section 4.1 Inclusion Criteria, pg. 62
The two study stratum will have different verbiage for major inclusion criteria.
Version 2.5 –Lifestyle will revert to inclusion criteria from version 2.3

Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (≥6 months ago), previous coronary bypass surgery (> 12 months ago), stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery or abnormal exercise tolerance test or an area of reversible ischemia on nuclear imaging study or pharmacologic stress, with subsequent revascularization, or angioplasty, or abnormal exercise treadmill stress test with or without nuclear imaging or echocardiography with the following exclusions:

Version 2.4-Salsalate will maintain the inclusion criteria from version 2.4

Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (≥6 months ago), previous coronary bypass surgery (> 12 months ago), or angioplasty, stable angina, or evidence of coronary artery disease on prior imaging studies including, plaque in at least one coronary artery, or abnormal exercise tolerance test, or an area of reversible ischemia on nuclear imaging study or pharmacologic stress, with subsequent revascularization or determined not to require intervention by care providing cardiologist, or abnormal exercise treadmill stress test with or without nuclear imaging or echocardiography with the following exclusions:

Note: the difference in these criteria includes the phrase “or determined not to require intervention by care providing cardiologist” We note that some patients may have known coronary artery disease associated with electrical or perfusion abnormalities on stress test and/or nuclear imaging that are medically treated and deemed medically stable and not requiring revascularization procedures at the current time. We will include these patients in the salsalate stratum of the study. The lifestyle stratum employs unsupervised exercise and will exclude patients with any evidence of reversible ischemia.

• Section 4.2 Exclusion Criteria, pg. 63

Criterion #8 Persons with allergies to contrast dye was deleted because is is the same as criterion #10

• Section 4.2 Exclusion Criteria, pg. 64

The following criterion was moved from the general exclusion criteria to the Salsalate only exclusion criteria.

History of significant chronic rheumatologic or other chronic inflammatory disease (including foot ulcers).

• Section 6.2.4.1 Expected Risks, pg. 73

The following procedure has been added for monitoring renal function in Salsalate patients per the recommendation of the data safety monitoring board.

In addition to the increased frequency of assessing urinary microalbumin, and in accordance with the recommendations of the data safety monitoring board for this study, any participant who had a baseline MCR <150 mcg/mg cr, who develops a microalbumin to creatinine ratio (MCR)
of 300mcg/mg cr or greater will have the laboratory assessment repeated with an unscheduled visit and for sustained elevation be evaluated by a nephrologist. For participants who entered the study with a baseline MCR > 150 mcg/mg cr we will recommend a nephrology consultation for a sustained doubling of the MCR rather than using a set cutoff value. Additionally, for participants with a large change, as defined above, discontinuation of the study drug will be strongly considered. All attempts will be made to keep the subject in the trial and evaluate the renal function after study drug discontinuation.

Protocol Amendment: Version 2.4

- Section 2.1 page 44

The inclusion criteria for normal renal function has been edited to include participants with a serum creatinine <1.3 if CrCl is < 60 ml/min/1.73m2 and there is no history of renal disease.

Version 2.3

In addition, subjects must be: a) aged 21-75 years inclusive; b) have BMI \( \leq 35 \text{ kg/m}^2 \) if females and \( \leq 40 \text{ kg/m}^2 \) if males; c) on a stable dose of an HMG CoA reductase inhibitor (statin) for 1 month at screening or unable to tolerate a statin; d) have normal renal function (note estimated creatinine clearance (eCrCL) calculated using Cockcroft-Gault (CG) [292] equation \( \geq 60 \) at screening \( \text{eCrCL}_{CG} \text{ (ml/min)} = \frac{(140 – \text{age}) \times \text{weight (kg)})}{\text{SCr (mg/dl)} \times 72} \times [0.85 \text{ if female}] \)

Version 2.4

In addition, subjects must be: a) aged 21-75 years inclusive; b) have BMI \( \leq 35 \text{ kg/m}^2 \) if females and \( \leq 40 \text{ kg/m}^2 \) if males; c) on a stable dose of an HMG CoA reductase inhibitor (statin) for 1 month at screening or unable to tolerate a statin; d) have normal renal function (note estimated creatinine clearance (eCrCL) calculated using Cockcroft-Gault (CG) [292] equation \( \geq 60 \) at screening \( \text{eCrCL}_{CG} \text{ (ml/min)} = \frac{(140 – \text{age}) \times \text{weight (kg)})}{\text{SCr (mg/dl)} \times 72} \times [0.85 \text{ if female}] \) or allowing inclusion of subjects with a serum creatinine of <1.3 if CrCl is < 60 ml/min/1.73m2 and there is no history of renal disease

Section 2.1 Screening Visit, page 45

If a subject has had a colonoscopy (negative for CA) in last 3 years, we will waive the stool guaiac/DRE requirement and we will attempt to obtain a recent EKG (within one year) from the patient’s provider to establish a baseline. If an EKG cannot be obtained at screening, the EKG performed at visit 2 will serve as a baseline

Version 2.3

The screening visit will include a medical history, physical exam, height, weight, waist circumference measurements, vital signs, screening lab panel (fasting glucose, Chem profile, CBC, Lipid panel, TSH and urine microalbumin/creatinine) and rectal exam for stool guaiac. (Latter for salsalate trial only).

Version 2.4

The screening visit will include a medical history, physical exam, height, weight, waist circumstance measurements, vital signs, screening lab panel (fasting glucose, Chem profile, CBC, Lipid panel, TSH and urine microalbumin/creatinine) and rectal exam for stool guaiac.
(Latter for salsalate trial only.) If the subject has had a colonoscopy (negative for CA) in the last 3 years, no stool guaiac/digital rectal exam (DRE) will be required. At this time, we will obtain an EKG performed within one year from the patient’s provider.

If a baseline EKG cannot be obtained at screening, the EKG performed at visit 2 will be used to establish a baseline.

**Section 2.2.2, page 49**

The guideline for baseline and end of treatment evaluations has been edited to state that patient in the salsalate stratum will return to the Joslin Diabetes Center for evaluation.

**Version 2.3**

Subjects will present to the General Clinical Research facility at the Beth Israel Deaconess Medical Center after a 12-hour fast.

**Version 2.4**

Subjects will present to the General Clinical Research facility at the Beth Israel Deaconess Medical Center after a 12-hour fast, or the Clinical Research Center of the Joslin Diabetes Center for the salsalate stratum.

**Section 2.3.3, page 52**

For participants who leave the study early but have completed one year or more following randomization, the MDCTA will be repeated.

**Version 2.3**

All attempts will be made to schedule an early termination visit for all subjects with non-urgent need to drop out of the trial (such as: relocating out of the geographical area). Early termination visits will occur in an identical manner to the end of treatment evaluation.

**Version 2.4**

All attempts will be made to schedule an early termination visit for all subjects with non-urgent need to drop out of the trial (such as: relocating out of the geographical area). Early termination visits will occur in an identical manner to the end of treatment evaluation. MDCTA will be repeated for participants who have completed one year or more of the trial following randomization.

2.3.6, page 55

**Section 2.3.6 is now the outline for early termination visits and the “Evaluation for Potential Toxicity & Side Effects Associated with Salsalate” section is numbered 2.3.7.**

**Version 2.3**

2.3.6 Evaluation for Potential Toxicity & Side Effects Associated with Salsalate

**Safety measures:** As discussed above, subjects will have a complete medical history and physical examination at the screening visit, baseline (prior to dispensing the drug/placebo) and end of treatment, and interim every 6 months throughout the study. Vital signs will be assessed
at each visit. Laboratory safety measures (chemistry, including Na, K, CL, Bicarb, Bun, Cr, ALT, AST, LDH, Alk phos, Total Bili, Ca, Phos, Uric Acid, Total Pro and Alb) will be measured at screening, prior to randomization and at 6 weeks, 3, 6, 9, 12, 18, 24, and 30 months during drug/placebo administration for the salsalate and placebo arms of the study and at 12, 24 and 30 months for the lifestyle trial of the study. Complete blood cell counts will be performed at baseline, 12, 24 and 30 months. Adverse events will be assessed at each visit and by phone at 6 weeks, 3, 6, 9, 12, 18, 24 and 30 months. Subjects who report adverse events during interim communications will be scheduled to meet with a study investigator.

Version 2.4

2.3.6 Early Termination
Study subjects will be encouraged to complete the study. However, all efforts will be made to collect all end of study measures for subjects seeking to withdraw participation prior to 30 months. All subjects who have had at least 12 months of participation following randomization, will be requested to undergo follow-up MDCTA.

2.3.7, page 55

For the participants with type 2 diabetes, HbA1c will be evaluated every six months to monitor glucose control in these patients. A urine microalbumin screening will also be added at every participant’s 6 month visit.

Version 2.3

Safety measures: As discussed above, subjects will have a complete medical history and physical examination at the screening visit, baseline (prior to dispensing the drug/placebo) and end of treatment, and interim every 6 months throughout the study. Vital signs will be assessed at each visit. Laboratory safety measures (chemistry, including Na, K, CL, Bicarb, Bun, Cr, ALT, AST, LDH, Alk phos, Total Bili, Ca, Phos, Uric Acid, Total Pro and Alb) will be measured at screening, prior to randomization and at 6 weeks, 3, 6, 9, 12, 18, 24, and 30 months during drug/placebo administration for the salsalate and placebo arms of the study and at 12, 24 and 30 months for the lifestyle trial of the study. Complete blood cell counts will be performed at baseline, 12, 24 and 30 months. Adverse events will be assessed at each visit and by phone at 6 weeks, 3, 6, 9, 12, 18, 24 and 30 months. Subjects who report adverse events during interim communications will be scheduled to meet with a study investigator.

Version 2.4
**Safety measures:** As discussed above, subjects will have a complete medical history and physical examination at the screening visit, baseline (prior to dispensing the drug/placebo) and end of treatment, and interim every 6 months throughout the study. Vital signs will be assessed at each visit. Laboratory safety measures (chemistry, including Na, K, CL, Bicarb, Bun, Cr, ALT, AST, LDH, Alk phos, Total Bili, Ca, Phos, Uric Acid, Total Pro and Alb) will be measured at screening, prior to randomization and at 6 weeks, 3, 6, 9, 12, 18, 24, and 30 months during drug/placebo administration for the salsalate and placebo arms of the study and at 12, 24 and 30 months for the lifestyle trial of the study. Urinary albumin will be assessed at 6, 12, 18, 24 and 30 months, HbA1c will be followed at the same intervals only in patients with established diabetes. Complete blood cell counts will be performed at baseline, 12, 24 and 30 months. Adverse events will be assessed at each visit and by phone at 6 weeks, 3, 6, 9, 12, 18, 24 and 30 months. Subjects who report adverse events during interim communications will be scheduled to meet with a study investigator.

**Table 14:** Laboratory Schedule for Salsalate and Placebo Arms

<table>
<thead>
<tr>
<th>Visit Number</th>
<th>Screening</th>
<th>Baseline</th>
<th>Active Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>-4 months to -1 week</td>
<td>0</td>
<td>Week 6</td>
</tr>
<tr>
<td>Salicylates</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>HbA1c</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Lipid Profile (Chol. Trig, LDL-C, HDL-C)</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-peptide</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemistry profile</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CBC with automated differential</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine microalbumin/creatinine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*(Chem profile: NA, K, CL, Bicarb, Bun, Cr, ALT, AST, LDH, Alk phos, Total Bili, Ca, Phos, Uric Acid, Total Pro, Alb)*

* Laboratory evaluation 2-4 weeks after end of 30-months treatment will be performed in subjects who fulfill either of the following conditions:

a) At the final dosing visit a decline in eGFR by 40 ml/min from baseline or a serum creatinine > 1.5 mg/dl in women and 1.6 in men.

b) At the final dosing visit a systolic blood pressure >160, a diastolic blood pressure >95, or a change in systolic or
Section 3.5, page 59

**Two per protocol secondary analyses as well as several secondary outcomes have been added to section 3.5.**

Version 2.3

### 3.5 Secondary Outcomes:

Important secondary outcomes include:

1. Improvement in parameters of the metabolic syndrome assessed by measures by waist circumference, systolic and diastolic blood pressure, lipid profiles (total cholesterol, triglycerides, HDL and LDL), and abdominal adiposity quantitated by computerized tomography.

2. Reduction of mediators of inflammation in the circulation including CRP, PAI-1, serum amyloid A, MMP-9 and fibrinogen, pro-inflammatory cytokines including IL-6, TNF-a and IL-1b, the adhesion molecules VCAM-1 and ICAM-1, increase in adiponectin and reduction in serum nitrotyrosine as a marker of oxidative stress.

3. Reduction of insulin resistance assessed by fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR).

4. Reduction of inflammation in the liver associated with nonalcoholic steatohepatitis (NASH), a newly recognized component of the metabolic syndrome, and reduction of fatty liver quantitated by computerized tomography and levels of AST and ALT as markers of liver inflammation related to NASH.
5) Comparison of rates of addition of anti-hypertensive, diabetic, or lipid lowering medication.
6) Comparison of numbers of persons with metabolic syndrome who progress to diabetes between groups.
7) Comparison of numbers of persons who regress from ATP III metabolic syndrome criteria. 
   (for those with metabolic syndrome).
8) Investigation of the relationship between vitamin D status and coronary calcification, as well 
   as with insulin resistance (HOMA-IR), beta-cell function (HOMA-%beta), and serum levels 
   of inflammatory cytokines and adhesion molecules, known to be related to CVD risk.
9) Determination of whether baseline vitamin D levels predict clinical response to salsalate, 
   and whether hypovitaminosis D is associated with plaque progression.

Version 2.4

3.5 Secondary Analysis and Outcomes:

Secondary Analysis:
Secondary analysis in the salsalate stratum will include two per protocol analyses, one with 
those on full dose and one including maximum tolerated dose to better understand both efficacy 
and safety finding related to drug exposure.

Secondary Outcomes
Important secondary outcomes include:

Coronary artery plaque assessments:
1) Baseline and change over 30 months in percent atheroma volume (PAV) calculated as the 
   proportion of the entire vessel wall occupied by atherosclerotic plaque; and total atheroma 
   volume, normalized to segment length.
2) Baseline and change over 30 months in maximum percent diameter stenosis and minimal 
   luminal diameter.
3) Comparisons of number and subjects with categorical variables of maximal stenosis >50% 
   and number with 3-vessel disease >20%.
4) Baseline and change over 30 months in the remodeling index will be calculated by the ratio 
   of plaque volume at the most diseased site compared to the least diseased site within the 
   proximal 10 mm of vessel at baseline and end of study.

Metabolic measures:
10) Improvement in parameters of the metabolic syndrome assessed by measures by waist 
circumference, systolic and diastolic blood pressure, lipid profiles (total cholesterol, 
   triglycerides, HDL and LDL), and abdominal adiposity quantitated by computerized 
tomography.
11) Reduction of mediators of inflammation in the circulation including CRP, PAI-1, serum 
amyloid A, MMP-9 and fibrinogen, pro-inflammatory cytokines including IL-6, TNF-a and IL-
   1b, the adhesion molecules VCAM-1 and ICAM-1, increase in adiponectin and reduction in 
   serum nitrotyrosine as a marker of oxidative stress.
12) Reduction of insulin resistance assessed by fasting insulin or C-peptide and homeostasis 
    model assessment of insulin resistance (HOMA-IR).
13) Reduction of inflammation in the liver associated with nonalcoholic steatohepatitis (NASH), 
    a newly recognized component of the metabolic syndrome, and reduction of fatty liver
quantitated by computerized tomography and levels of AST and ALT as markers of liver inflammation related to NASH.

14) Comparison of rates of addition of anti-hypertensive, diabetic, or lipid lowering medication.

15) Comparison of numbers of persons with metabolic syndrome who progress to diabetes between groups.

16) Comparison of numbers of persons who regress from ATPIII metabolic syndrome criteria. (for those with metabolic syndrome).

17) Investigation of the relationship between vitamin D status and coronary calcification, as well as with insulin resistance (HOMA-IR), beta-cell function (HOMA-%beta), and serum levels of inflammatory cytokines and adhesion molecules, known to be related to CVD risk.

18) Determination of whether baseline vitamin D levels predict clinical response to salsalate, and whether hypovitaminosis D is associated with plaque progression.

Section 4.1, page 62

The wording of the inclusion criteria for established coronary artery disease will be amended to include patients who have strong evidence of CAD on prior imaging studies or, present with major risk factors as well as supporting imaging via chest CT.

Version 2.3

Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (≥6 months ago), previous coronary bypass surgery (> 12 months ago), stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery or abnormal exercise tolerance test or an area of reversible ischemia on nuclear imaging study or pharmacologic stress, with subsequent revascularization, or angioplasty, or abnormal exercise treadmill stress test with or without nuclear imaging or echocardiography with the following exclusions:

Version 2.4

Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (≥6 months ago), previous coronary bypass surgery (> 12 months ago), or angioplasty, stable angina, or evidence of coronary artery disease on prior imaging studies including, plaque in at least one coronary artery, or abnormal exercise tolerance test, or an area of reversible ischemia on nuclear imaging study or pharmacologic stress, with subsequent revascularization or determined not to require intervention by care providing cardiologist, or abnormal exercise treadmill stress test with or without nuclear imaging or echocardiography with the following exclusions:

Section 4.2, page 64

The exclusion criteria have been amended so that potential subjects with mild tinnitus (defined as less than or equal to 4/10 in intensity) will be allowed to participate in the study. Patients with severe tinnitus (5/10 or greater in intensity) will still be excluded from participating in the study.

Version 2.3

Exclusions Specific to Salsalate Trial
Chronic tinnitus
Version 2.4
Exclusions Specific to Salsalate Trial
Chronic, severe tinnitus (pt self report of 5/10 or greater intensity). *
*Patients who report mild, chronic tinnitus (4/10 intensity or less) may be considered for participation

Section 5.2.2, page 65
Urinary albumin excretion has been added as a measure of renal function for patients who develop adverse GI effects while on salsalate.

Version 2.3
Salsalate will be stopped for development of guiac positive stool or fall in hematocrit to < 2 standard deviations below the lower limit. Subjects could develop allergic reactions to the drug during treatment, although this is rare. Renal function will be monitored by use of estimated glomerular filtration rates

Version 2.4
Salsalate will be stopped for development of guiac positive stool or fall in hematocrit to < 2 standard deviations below the lower limit. Subjects could develop allergic reactions to the drug during treatment, although this is rare. Renal function will be monitored by use of estimated glomerular filtration rates and urinary albumin excretion.

Protocol Amendment: Version 2.3
• Section 5.2.4. page 65
The metoprolol dosing prior to MDCTA was revised to conform to the current radiology protocol.

Version 2.2
For subjects with a heart rate greater than this, the beta-blocker Metoprolol (50 mg) will be administered by mouth to reduce the rate to approximately 60 beats per minute one hour before the MDCTA. If the subject's heart rate remains greater than 65 beats per minute at 1 hour after 50 mg of metoprolol is given by mouth, additional 5 mg metoprolol will be given every 5 minutes up to 3 times or until the heart rate goes down to 65 beats per minute.

Version 2.3
For subjects with a heart rate greater than this, the beta-blocker Metoprolol (50 mg) will be administered by mouth to reduce the rate to less than 65 beats per minute one hour before the MDCTA. If the subject's heart rate remains greater than 65 beats per minute at 1 hour after 50 mg of metoprolol is given by mouth, additional metoprolol will be given per the current radiology protocol until the heart rate goes down to 65 beats per minute.

Patients with macroalbuminuria (urine microalbumin/creatinine >300 mcg/mg cr) will be excluded from the salsalate study, therefore urine microalbumin/creatinine test was moved from visit 2 to visit 1 and urine microalbumin/creatinine >300 mcg/mg cr at
screening was added to salsalate exclusion criteria. The changes regarding the timing of urine microalbumin/creatinine and addition of exclusion criterion are in the following sections:

- Section 2.1, page 45; Section 2.2.2, page 48; Section 2.3.3, page 51; and tables 13 and 14 on pages 50 and 53 respectively:

Version 2.2
fasting glucose, Chem profile, CBC, Lipid panel and TSH

Version 2.3
fasting glucose, Chem profile, CBC, Lipid panel, TSH and urine microalbumin/creatinine

- Section 5.2.4. page 65

Version 2.3
Urine microalbumin/creatinine > 300 mcg/mg cr.

In order to increase salsalate recruitment, based on recommendation of the DSMB, the BMI criterion was modified so that for both lifestyle and salsalate patients BMI 26.6 is rounded to 27 and BMI of 25 to 26.5 is inclusionary with an elevated C-reactive protein or with increased waist circumference. These changes are in:

- Section 2.1, page 44 and section 4.1, page 60

Version 2.2
In addition, subjects eligible for the salsalate trial must also have a BMI of ≥ 27.0 kg/m² as a surrogate of the metabolic syndrome and subjects eligible for the lifestyle study must either have a BMI of >27 kg/m² (26.6 is rounded to 27) as a surrogate of the metabolic syndrome or a BMI of 25 to 26.5 with either 2 additional components of the metabolic syndrome or an elevated C-reactive protein.

In addition, subjects must be: a) aged 21-75 years inclusive; b) have BMI ≥27 kg/m² and ≤35 kg/m² if females and ≤40 kg/m² if males (to prevent under-representation of minority persons in our investigations, we will use a BMI ≥24.5 for subjects from Asian origin [291]);

Version 2.3
Subjects must also have either a BMI of ≥ 27 kg/m² (26.6 is rounded to 27) as a surrogate of the metabolic syndrome or a BMI of 25 to 26.5 with an elevated C-reactive protein or with increased waist. Assessment of increased waist will be determined based on the criteria described in “Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and international association for the Study of Obesity” guidelines, or for lifestyle with 2 additional components of metabolic syndrome. (to prevent under-representation of minority persons in our investigations, we will use a BMI ≥24.5 for subjects from Asian origin [291])

In addition, subjects must be: a) aged 21-75 years inclusive; b) have BMI ≤ 35 kg/m² if females and ≤40 kg/m² if males

- Section 2.1, page 45

Version 2.2
CVD patients with a BMI of ≥ 27.0 would be scheduled for a fasting screening at a local Quest site after being consented at the cardiologist’s office.
Version 2.3
CVD patients would be scheduled for a fasting screening at a local Quest site after being consented at the cardiologist’s office.

The time allowed between Screening and Baseline was increased to 4 months. This change can be seen at:

Section 2.1 pages 43, 44 and 45; section 2.2.2, page 48; section 2.3 page 51; section 2.3.3 page 52; and tables 12, 13 and 14 pages 43, 50 and 51
Protocol Amendment: Version 2.2

- Section 1.3, page 5:
  Correction of the randomization ratio.

  Version 2.1
  Randomization will occur in a 1:1:1 ratio of lifestyle to standard of care to and salsalate to placebo. The placebo arm will follow a schedule similar to that of the salsalate.

  Version 2.2
  Randomization will occur in a 1:1 ratio of lifestyle to standard of care and salsalate to placebo.

- Section 3.4, page 59:
  Clarification and correction in the Sample Size Determination portion.

  Version 2.1
  This preliminary data showed a 9.3% decrease in LDL cholesterol

  Version 2.2
  The lifestyle preliminary data showed a 9.3% decrease in LDL cholesterol

  and

  Version 2.1
  …this study will have a power of 0.80 to detect a 4% difference in rate of plaque progression between active and placebo/control

  Version 2.2
  …this study will have a power of 0.80 to detect a 3.9% difference in rate of plaque progression between active and placebo/control

- Section 3.6, page 60:
  Version 2.1
  The primary analytic approach will be analysis of variance (ANOVA) using Dunnett’s test to compare each of the active treatment groups to their respective standard of care /placebo.

  Version 2.2
  The primary analytic approach will be to use student’s t-test to compare each of the active treatment groups to their respective standard of care /placebo group.
Protocol Amendment: Version 2.1

This amendment provides further clarification that due to the more rigorous exclusion criteria for salsalate, patients potentially eligible for salsalate will first be offered this trial and only if they are not eligible for salsalate or not willing to participate in the salsalate trial, they will be offered the lifestyle trial

- Section 1.2, page 4:
The sentence saying "Patients will have the choice of either the lifestyle trial or the salsalate trial" was deleted

Section 2.1, page 43:
Version 2.0
Since the exclusion criteria are stricter for salsalate trial, those patients eligible for salsalate will have the choice of entering it and being randomized to salsalate or placebo. If they prefer lifestyle, they can enter lifestyle. Patients not eligible for salsalate but eligible for lifestyle will be randomized to the lifestyle intervention or usual care.

Version 2.1
Since the exclusion criteria are stricter for salsalate trial, those patients potentially eligible for salsalate will be screened for salsalate and if eligible will be randomized to salsalate or placebo. If they are not eligible for salsalate or decline the salsalate study, they will be offered the lifestyle study. Patients not eligible for salsalate but eligible for lifestyle will be randomized to the lifestyle intervention or usual care.

Section 2.1, page 46:
The section about enrollment projection from version 2.0 was deleted as it is not relevant to the protocol, rather specific for the study timeline:

Of the 680 total expected to be enrolled, we have enrolled 30 patients already; therefore, the remaining 650 will be enrolled over 18 months for 17 per month for lifestyle and 17 per month for salsalate. We may need 24 months for enrollment for 26 per month: 13 for lifestyle and 13 for salsalate. For randomization, we need to randomize 546 additional (10 have been randomized). This is 15 per month for lifestyle and 16 per month for salsalate over 18 months and 11 per month for lifestyle and 11 per month for salsalate over a 24 month period.

Section 3.4, page 59 was modified to clarify the sample size determination

Version 2.0
The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. This preliminary data showed a 9.3% decrease in LDL cholesterol [182]. As the majority of the patients in this trial will be on statin therapy, we expect a 30% smaller treatment effect, resulting in a reduction in LDL cholesterol of 6.5%. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24 ±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol [304]. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume [305, 306], this trial expects to observe a 3.9% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group and an average of 1.7 measurable plaques per study subject, the number of subjects required to have a type II error of 0.2 (80% power) with a type I error of 0.05 (alpha 0.05) is 104 per treatment arm. This analysis assumes that plaque volume changes are independent within each study subject. Data from prior invasive angiographic trials suggests that this is not the case, with an absolute correlation of up to 0.24[307]. Assuming that the intra-subject correlation is 0.24, the appropriate variance inflation factor is 1.06 to account for this.
lack of statistical independence [308]. Thus, a total of 111 subjects is required for each treatment arm. With an initial randomization of 139 subjects to each arm and a 20% dropout rate, resulting in a total of 111 subjects with assessment of the primary endpoint, this study will have a power of 0.80. To account for our 18% screen and baseline failure rate, we will enroll 170 per arm per trial (680 total for both trials).

Version 2.1
The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. There is no direct preliminary data available regarding the effects of salsalate therapy on coronary atherosclerosis. This preliminary data showed a 9.3% decrease in LDL cholesterol [182]. As the majority of the patients in this trial will be on statin therapy, we expect a 30% smaller treatment effect, resulting in a reduction in LDL cholesterol of 6.5%. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol in 27 patients [304]. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume [305, 306], this trial expects to observe a 3.9% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group and an average of 1.7 measurable plaques per study subject, the number of subjects required to have a type II error of 0.2 (80% power) with a type I error of 0.05 (alpha 0.05) is 104 per treatment arm. This analysis assumes that plaque volume changes are independent within each study subject. Data from prior invasive angiographic trials suggests that this is not the case, with an absolute correlation of up to 0.24[307]. Assuming that the intra-subject correlation is 0.24, the appropriate variance inflation factor is 1.06 to account for this lack of statistical independence [308]. Thus, a total of 111 subjects is required for each treatment arm. With an initial randomization of 139 subjects to each arm and a 20% dropout rate, resulting in a total of 111 subjects with assessment of the primary endpoint, this study will have a power of 0.80 to detect a 4% difference in rate of plaque progression between active and placebo/control. To account for our 18% screen and baseline failure rate, we will enroll 170 per arm per trial (680 total for both trials).

Section 3.6, page 60 and Chapter 6, page 69:
Clarification that each study active arms will be compared to their respective standard of care and placebo

Version 2.0
The primary analytic approach will be analysis of variance (ANOVA) using Dunnett’s test to compare the active treatment groups to placebo.

Version 2.1
The primary analytic approach will be analysis of variance (ANOVA) using Dunnett’s test to compare each of the active treatment groups to their respective standard of care/placebo.

And

Version 2.0
The overall goal of this project is to compare the effect of salsalate and an intensive lifestyle intervention geared to weight loss to usual care

Version 2.1
The overall goal of this project is to compare the effect of salsalate and an intensive lifestyle intervention geared to weight loss to usual care/placebo

Section 4.2, page 62:
The exclusion criterion of asthma was clarified:
Version 2.0
History of asthma
Version 2.1
History of asthma only if unable to tolerate beta-blockers
Section 4.2, page 63:
**Patient with history of gastric bypass surgery will not eligible for the salsalate study**
Version 2.1
17) History of gastric bypass surgery
Protocol Amendment: Version 2.0

A. The study protocol had been split into two separate parallel trials:

1) Salsalate intervention with a salsalate-placebo control

2) Lifestyle intervention with the usual care control.

The exclusion criteria for the two trials will not be identical and given that the exclusion criteria for the salsalate trial are more rigorous, patients eligible for salsalate will first be offered this intervention and if they are not willing to participate or not eligible for salsalate, they will be offered the lifestyle trial. These changes are in the following sections:

- Section 1.2, page 4:

Version 1.9
The TINSAL-CVD study is a randomized, double-masked, placebo-controlled, 3 arm, clinical trial. The purpose of the study is to compare the effect of lifestyle intervention, salsalate or placebo on sub-acute inflammation and coronary plaque,

Version 2.0
Patients will have the choice of either the lifestyle trial or the salsalate trial. The salsalate trial will be a randomized, double-masked, placebo-controlled, clinical trial. In the lifestyle trial, patients will be randomized to lifestyle plus four fish oil capsules daily or current standard of care. The purpose of the study is to compare the effect of lifestyle intervention and salsalate to their respective standard of care and placebo on sub-acute inflammation and coronary plaque,

- Section 1.3, page 5:

Version 1.9
...with three parallel arms: one with lifestyle modification and Lovaza (previously known as Omacor, omega 3 supplementation), a prescription medication available in the US only with a physician’s prescription, the second with IKKβ/NF-κB inhibition using salsalate, and the third with usual care and or salsalate placebo. Coronary plaque remodeling by each intervention will be assessed by MDCTA. Randomization will occur in a 1:1:1 ratio of lifestyle to salsalate to placebo. The placebo arm will follow a schedule similar to that of the salsalate.

Version 2.0
There are two separate trials: one with lifestyle modification and Lovaza (previously known as Omacor, omega-3 supplementation), a prescription medication available in the US only with a physician’s prescription, and the second trial with IKKβ/NF-κB inhibition using salsalate. Since the exclusion criteria are stricter for salsalate trial, those patients eligible for salsalate will have the choice of entering it and being randomized to salsalate or placebo. If they prefer lifestyle, they can enter lifestyle. Patients not eligible for salsalate but eligible for lifestyle will be randomized to the lifestyle intervention or usual care. Coronary plaque remodeling by each intervention will be assessed by MDCTA.

- Section 1.3, page 5:

Version 1.9
Coronary plaque will be measured before and following a 30 month intervention in 3 arms:

1) Intensive Lifestyle intervention including: Intensive dietary and exercise program to get people to a BMI of 25 or less and around 1 hour of daily exercise.

2) Treatment with Salsalate (Disalcid™) 3.5 grams a day.

3) Standard of care with salsalate placebo.
Specific aims will determine whether intensive lifestyle intervention, which includes the daily use of Lovaza, a prescription medication available in the US only with a physician’s prescription, compared to usual care or salsalate treatment, compared to the usual care, in patients with CVD and metabolic syndrome on statins will:

Version 2.0
Coronary plaque will be measured before and following a 30 month intervention in all patients.

Specific aims will determine whether intensive lifestyle intervention, which includes the daily use of Lovaza, a prescription medication available in the US only with a physician’s prescription, compared to usual care or salsalate treatment, compared to placebo in overweight/obese patients with CVD will:

- Chapter 2, page 45:

Version 1.9
The study is a multi-center, prospective, interventional design. There are three arms including intensive lifestyle interventions salsalate and salsalate placebo. It is recognized there is no true masking for participation in a diet and exercise program, however the salsalate arm will be double masked and both groups will be compared to salsalate placebo. Therefore, the protocol will first be presented for all arms jointly and afterwards the lifestyle arm and the salsalate will be described separately in more details. The placebo arm will be conducted identically to the salsalate arm.

2.1 Study Design
This is a 30-month, randomized, placebo-controlled, parallel-groups study with a 3 month screening period and a 30-month treatment period. While the lifestyle arm cannot be blinded, the salsalate and the placebo arms will be double-blinded. There will be a total of 10 visits. The baseline and final evaluation will be performed at the General Clinical Research Center (GCRC) of the Beth Israel Deaconess Medical Center (BIDMC)/Joslin Diabetes Center (JDC). Interim visits will occur in the office of the patient’s cardiologist care provider, or at BIDMC/JDC.

Version 2.0
The study is a multi-center, prospective, interventional design. There are two trials: one randomizing patients to intensive lifestyle intervention or usual care and the second randomizing patients to salsalate or salsalate placebo. It is recognized there is no true masking for participation in a diet and exercise program, however the salsalate trial will be double masked and both groups will be compared to their respective usual care or placebo. Therefore, the protocol will first be presented for both trials jointly and afterwards the lifestyle trial and the salsalate trial will be described separately in more details.

2.1 Study Design
The study is a trial of overweight/obese patients with known stable coronary heart disease. There are two separate trials: one with lifestyle modification and Lovaza, a prescription medication available in the US only with a physician’s prescription, and the second with IKKβ/NF-κB inhibition using salsalate. Since the exclusion criteria are stricter for salsalate trial, those patients eligible for salsalate will have the choice of entering it and being randomized to salsalate or placebo. If they prefer lifestyle, they can enter lifestyle. Patients not eligible for salsalate but eligible for lifestyle will be randomized to the lifestyle intervention or usual care. This is a 30-month study with a 3 month screening period and a 30-month treatment period. While the lifestyle arm and its usual care control cannot be blinded, the salsalate and the placebo arms will be double-blinded. There will be a total of 7 visits (with 3 additional phone and blood test visits for the salsalate trial). The baseline and final evaluation will be performed at the General Clinical Research Center (GCRC) of the Beth Israel Deaconess Medical Center.
Interim visits will occur in the office of the patient’s cardiologist care provider, or at BIDMC/JDC.

- Section 2.1 page 48:
  Version 1.9
  Patients qualifying for the study by the results of their screening visit MDCTA will be randomized to lifestyle salsalate or placebo in a 1:1:1 ratio.
  
  Version 2.0
  Patients qualifying for the study by the results of their screening visit MDCTA will be randomized to the active or usual care or placebo arm in either the lifestyle or salsalate trial.

- Section 2.1 page 49:
  Version 1.9
  Once a patient is assigned to a specific study arm, the patient is then followed by that arm’s study nurse or coordinator and that arm’s principal investigator (PI) for the life of the study. Patients assigned to other than lifestyle intervention will not know to which arm of the study they have been assigned. The PI will know whether they are getting active lifestyle intervention but not whether they are receiving salsalate or placebo.
  
  Version 2.0
  Once a patient is assigned to a specific study trial, the patient is then followed by that study nurse or coordinator and that study principal investigator (PI) for the life of the study. Patients assigned to other than lifestyle intervention or usual care will not know to which arm of the study they have been assigned. The PI will know whether they are getting active lifestyle intervention or usual care but not whether they are receiving salsalate or placebo.

B. The salsalate trial of the study will be conducted at the Joslin Diabetes Center with Dr. Allison Goldfine the principle investigator. The Lifestyle trial will be conducted at Beth Israel Deaconess Medical Center with Dr. Francine Welty the principle investigator. Related changes are on pages: 73, 74, 78 and 79.

C. The Metabolic Syndrome is no more required in the inclusion criteria. Patients will be overweight/obese (BMI ≥27.0 kg/m²). Although fulfillment of 3 out of 5 metabolic syndrome criteria is not required any more for inclusion in the study, we will still follow component of the metabolic syndrome as part of our secondary outcome measures. Therefore the term metabolic syndrome was deleted across the protocol with the term overweight/obese replacing it:

- Title and top of page 4:
  Version 1.9
  Targeting INflammation using SAlsalate or Lifestyle intervention in the Metabolic Syndrome and CardioVascular Disease
  
  Version 2.0
  Targeting INflammation using SAlsalate or Lifestyle intervention in CardioVascular Disease

- Section 1.1, page 4:
  Version 1.9
  Insulin resistance and the metabolic syndrome appear to be at the root of the pathogenic process.
  
  Version 2.0
  Obesity induced insulin resistance appear to be at the root of the pathogenic process.
Section 1.1, page 4; section 1.3 pages 5 and 6; section 2.1 section 4; section 2.3 page 54; section 2.3.5 page 58; section 5.1 page 69; chapter 6 page 73:

Version 1.9
Patients with the metabolic syndrome and established CVD

Version 2.0
Overweight/obese patients with established CVD

Section 2.1, page 46:

In addition, subjects must also meet the ATPIII components of the metabolic syndrome which defines metabolic syndrome as the presence of any 3 of 5 clinical diagnostic traits: 1) abdominal girth > 35 inches (88 cm) in women, > 40 inches (102 cm) in men; 2) HDL-C < 40 mg/dL in men and < 50 mg/dL in women; 3) fasting triglyceride > 150 mg/dL (1.69 mmol/L) or triglycerides > 125 mg/dl (1.4 mmol/L) on concomitant statin therapy; 4) blood pressure ≥ 130/85 mm Hg or treated hypertension; and 5) fasting glucose ≥ 100 mg/dL (>6.1 mmol/L). For consistency, we have adopted this definition. However, as we recognize the importance of the metabolic syndrome in minority populations, and to prevent under-representation of minority persons in our investigations, we will use developed ethnic specific waist ratios.

Version 2.0
In addition, subjects eligible for the salsalate trial must also have a BMI of ≥ 27.0 kg/m² as a surrogate of the metabolic syndrome and subjects eligible for the lifestyle study must either have a BMI of >27 kg/m² (26.6 is rounded to 27) as a surrogate of the metabolic syndrome or a BMI of 25 to 26.5 with either 2 additional components of the metabolic syndrome or an elevated C-reactive protein.

Section 2.1, page 48:

CVD patients with metabolic syndrome

Version 2.0
CVD patients with a BMI of ≥ 27.0

Section 3.5, page 63:

Improvement in the metabolic syndrome

Version 2.0
Improvement in parameters of the metabolic syndrome

and

Comparison of numbers of persons with metabolic syndrome who progress to diabetes between groups.

Version 2.0
Comparison of numbers of persons who progress to diabetes between groups.

and

Comparison of numbers of persons who regress from ATPIII metabolic syndrome criteria

Version 2.0
Comparison of numbers of persons who regress from ATPIII metabolic syndrome criteria (for those with metabolic syndrome).

- Section 4.1, page 65:

Version 1.9
Subjects must also meet the ATPIII components of the metabolic syndrome: which defines metabolic syndrome as the presence of any 3 of 5 clinical diagnostic traits: 1) abdominal girth > 35 inches (88 cm) in women, > 40 inches (102 cm) in men; 2) HDL-C < 40 mg/dL in men and < 50 mg/dL in women or on treatment for low HDL; 3) fasting triglyceride > 150 mg/dL (1.69 mmol/L) or on treatment for high triglycerides; 4) blood pressure ≥ 130/85 mm Hg or treated hypertension; and 5) fasting glucose ≥ 100 mg/dL (>6.1 mmol/L). For consistency, we have adopted this definition. However, as we recognize the importance of the metabolic syndrome in minority populations, and to prevent under-representation of minority persons in our investigations, we will use developed ethnic specific waist ratios.

Version 2.0
Please note that the requirement for BMI of ≥ 27.0 kg/m² is a surrogate of the metabolic syndrome.

- Section 4.1, page 66:

Version 1.9
Medicare subjects are more likely to be diagnosed with the metabolic syndrome and coronary heart disease

Version 2.0
Medicare subjects are more likely to be diagnosed with coronary heart disease

D. The BMI criteria for the lifestyle patients was modified on pages 47 and 63:

Version 2.0
Subjects eligible for the lifestyle study must have either a BMI of ≥ 27 kg/m² (26.6 is rounded to 27) as a surrogate of the metabolic syndrome or a BMI of 25 to 26.5 with either 2 additional components of metabolic syndrome or an elevated C-reactive protein.

E. The analysis of the primary endpoint will be done based on per plaque analysis rather than per patients. These changes will affect also the study power calculation.

- Section 2.1, page 45:

Version 1.9
Approximately 1000 patients will be screened in this study. With an estimated 20% screen failure 800 will have baseline evaluation including MDCTA. With an estimated 10% of MDCTA that will be difficult to evaluate, 720 patients will be randomized in a 1:1:1 ratio to one of the three arms: lifestyle intervention, or salsalate or placebo, such that for the lifestyle arm 240 will be randomized to active dietary, exercise and nutritional supplementation (with omega-3 fatty acids in the form of Lovaza, a prescription medication available in the US only with a physician’s prescription), for the salsalate arm 240 will be randomized to active drug and for the placebo arm 240 will be randomized to usual care and salsalate placebo.

Version 2.0
Approximately 340 patients will be screened for the lifestyle trial and 390 for the salsalate trial. With an estimated 18% screen and baseline failure rate for lifestyle, 278 will be randomized in lifestyle with 139 to active dietary, exercise and nutritional supplementation (with omega-3 fatty acids in the form of Omacor (now known as Lovaza), a prescription medication available in the US only with a physician’s prescription), and 139 to usual care in the lifestyle trial. With an
estimated 30% screen and baseline failure rate for salsalate, 278 will be randomized in the salsalate trial: 139 to salsalate and 139 to salsalate placebo.

- Section 2.1, page 48:
  Version 1.9
  It is anticipated that 800 patients will undergo baseline visit and MDCTA and 720 patients will remain after 10% MDCTA failure: 240 in each arm of the study. Of the 680 total 720 patients will be enrolled over 2.5 years for about 24 patients per month at BIDMC/JDC.

  Version 2.0
  It is anticipated that 626 patients will undergo baseline visit and MDCTA and 556 patients will remain after MDCTA failure: 278 in lifestyle and 278 in salsalate. Of the 680 total expected to be enrolled, we have enrolled 30 patients already; therefore, the remaining 650 will be enrolled over 18 months for 17 per month for lifestyle and 17 per month for salsalate. We may need 24 months for enrollment for 26 per month: 13 for lifestyle and 13 for salsalate. For randomization, we need to randomize 546 additional (10 have been randomized). This is 15 per month for lifestyle and 15 per month for salsalate over 18 months and 11 per month for lifestyle and 11 per month for salsalate over a 24 month period.

- Section 2.3, page 54:
  Version 1.9
  Approximately 240 patients will be randomly assigned to salsalate and 240 patients to salsalate placebo.

  Version 2.0
  Approximately 139 patients will be randomly assigned to salsalate and 139 patients to salsalate placebo.

- Section 3.1, page 61:
  Version 1.9
  We expect to screen approximately 1000 subjects identified at cardiology clinics through cardiology sites and census mailing (note that patients seen in the cardiology clinics at the BIDMC will also be able to be screened and enrolled. If screen failures exceed the predicted 10-20% rate we will notify the Committee on Clinical Investigation. We hope to perform baseline evaluation on 800 subjects and anticipate that 10% of MDCTA scans will not be of adequate quality to permit longitudinal evaluation. Thus, 720 subjects will be enrolled in the three treatment arms of the study: 240 to lifestyle, 240 to salsalate and 240 to placebo.

  Version 2.0
  For the lifestyle trial we expect to screen approximately 340 subjects and for the salsalate trial we expect to screen approximately 389 subjects identified at cardiology clinics through cardiology sites and census mailing (note that patients seen in the cardiology clinics at the BIDMC will also be able to be screened and enrolled. If screen failures exceed the predicted 10-20% rate we will notify the Committee on Clinical Investigation. We hope to perform baseline evaluation on 313 subjects in the lifestyle trial and 313 subjects in the salsalate trial. We anticipate that 10% -11% of MDCTA scans will not be of adequate quality to permit longitudinal evaluation. Thus, 278 subjects will be enrolled in the lifestyle trial and 278 subjects in salsalate trial.

- Sections 3.3 and 3.4, page 62:
  Version 1.9

3.3 Primary Outcome:
The primary outcome will measure change in soft plaque measured by Computed Tomography (CT) angiography between baseline and 30 months.

3.4 Sample size determination

The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. This preliminary data showed a 9.3% decrease in LDL cholesterol. As the majority of the patients in this trial will be on statin therapy, we expect a 30% smaller treatment effect, resulting in a reduction in LDL cholesterol of 6.5%. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24 ±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume, this trial expects to observe a 4.2% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group, the number of subjects required to have a type II error of 0.2 (80% power) with a p-value of 0.05 is 176 per treatment arm. With an initial randomization of 240 subjects to each arm and a 20% dropout rate, resulting in a total of 192 subjects with assessment of the primary endpoint, this study will have a power of 0.83.

Version 2.0

3.3 Primary Outcome:

The primary outcome will measure change in soft plaque measured by Computed Tomography (CT) angiography between baseline and 30 months on a per plaque basis. This is a change from our original primary endpoint which was change in plaque volume per patient. The reason for the change is that data since our grant submission has shown that plaque progression and regression is greatly influenced by shear stress forces which vary from one plaque to another depending on the location of the plaque in the artery [302, 303].

3.4 Sample size determination

The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. This preliminary data showed a 9.3% decrease in LDL cholesterol [182]. As the majority of the patients in this trial will be on statin therapy, we expect a 30% smaller treatment effect, resulting in a reduction in LDL cholesterol of 6.5%. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24 ±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol [304]. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume [305, 306], this trial expects to observe a 3.9% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group and an average of 1.7 measurable plaques per study subject, the number of subjects required to have a type II error of 0.2 (80% power) with a type I error of 0.05 (alpha 0.05) is 104 per treatment arm. This analysis assumes that plaque volume changes are independent within each study subject. Data from prior invasive angiographic trials suggests that this is not the case, with an absolute correlation of up to 0.24 [307]. Assuming that the intra-subject correlation is 0.24, the appropriate variance inflation factor is 1.06 to account for this lack of statistical independence [308]. Thus, a total of 111 subjects is required for each treatment arm. With an initial randomization of 139 subjects to each arm and a 20% dropout rate, resulting in a total of 111 subjects with assessment of the primary endpoint, this study will have a power of 0.80. To account for our 18% screen and baseline failure rate, we will enroll 170 per arm per trial (680 total for both trials).

F. Women with child bearing potential will be allowed in the study if they agree to use effective contraceptive methods for the duration of the study. Urine βHCG will be measured before MDCTA to rule out pregnancy. This changes are on pages 47 and 65.
women of child bearing potential

if women are of child bearing potential they must have a pregnancy test prior to the CT angio and agree to use contraceptive methods for the duration of the study;

G. Recent ETT will no longer be required for the salsalate trial as increase in unsupervised exercise is no more part of the intervention. These changes are on pages 47 and 65.

H. Stool Guaiac is no more required for patient in the lifestyle trial (pages 48 and 51)

I. Some of the exclusion criteria will be trial specific (pages 67-68):

The exclusion of subject with iron storage disease is no more required as a new batch of Lovaza tablets not containing iron will be given.

Exclusions Specific to Salsalate Trial:
1) Prior hemorrhagic stroke
2) persons with known aspirin allergy
3) Use of continuous oral corticosteroid treatment (more than 2 weeks), or patients requiring corticosteroids within 3 months
4) Anti-diabetic medication including thiazolidinedione (pioglitazone or rosiglitazone), or insulin or Extendin-4 (Byetta)
5) History of peptic ulcer or gastritis within 5 years
6) Positive stool guaiac
7) Hemoglobin 2 standard deviations below normal
8) Low platelet count (2 standard deviations below normal)
9) Known bleeding disorder
10) Coumadin (warfarin compounds)
11) History of type 1 diabetes and/or history of ketoacidosis
12) Daily use of NSAIDS (including salsalate) for arthritis
13) History of malignancy, except subjects who have been disease-free for greater than 5 years, or whose only malignancy has been basal or squamous cell skin carcinoma
14) History of drug or alcohol abuse, or current weekly alcohol consumption >14 units/week (1 unit = 1 beer, 1 glass of wine, 1 mixed cocktail containing 1 ounce of alcohol)
15) Use of probenecid (Benemid, Probalan), sulfinpyrazone (Anturane) or other uricosuric agents
16) Chronic tinnitus.

Exclusions specific to Lifestyle Trial:
1) Prior stroke with residual cognitive deficit or functional deficit preventing any type of exercise
2) Current chemotherapy or radiation for malignancy
3) Current weekly alcohol consumption > 21 units/week (1 unit = 1 beer, 1 glass of wine, 1 mixed cocktail containing 1 ounce of alcohol

J. corrections/clarifications:

1. We are not measuring waist/hip ratio but rather waist circumference in this study. Changes are on pages 6, 48, 51, 55 and 63

2. Randomization will be stratified by the presence of diabetes and not by study site (page 61)

3. Oral or IV hydration is done after MDCTA rather than before. (pages 52 and 55)

4. Patients will be recommended to take aspirin 81-325 mg rather than 81 mg. (pages 49, 51, 55)

5. Study drug will be shipped every 4 months rather than every 6 months (page 53).

6. In the section about the risk of hypoglycemia we added that: (section 2.3.6 page 59)
   Weight loss and increased exercise can also improve insulin sensitivity and may predispose subjects on medications such as sulfonylurea or insulin to hypoglycemia.
Protocol Amendment: Version 1.9

GlaxoSmithKline, the manufacturer of Lovaza, requested the addition of Iron storage disease to the study exclusion criteria due to the presence of iron oxide in the capsules used for clinical studies. Therefore the diagnosis of iron storage disease was added to the study exclusion criteria on page 65.

We revised our protocol to include also patients who have had coronary bypass surgery. The MDCTA analysis will be based solely on plaque in native coronary arteries. We therefore deleted the coronary bypass surgery exclusion criteria (page 65) and added on pages 46 and 64 the following:

Version 1.8:
Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (> 6 months ago),

Version 1.9:
Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (> 6 months ago), previous coronary artery bypass surgery (> 12 months ago),

And on page 46:

Version 1.8:
Subjects who have had coronary artery bypass grafting or unstable angina are not eligible.

Version 1.9:
Subjects who have had coronary artery bypass grafting less than 12 months ago or unstable angina are not eligible.

We increased the upper age limit for enrollment to 75. Please see changes on pages 46 and 63.

In the amendments for version 1.5 we changed the screening period from 4 weeks to 3 months. To be in agreement with this change we made corrections on pages 47, 50, 52, 54 and 55 clarifying that the screening period is from -3 months to -1 week rather than week -12 to -1.
Protocol Amendment: Version 1.8

The salsalate dose will be reduced to 3.5 grams/day divided to one dose of 4 tablets and one dose of 3 tablets. This amendment is done based upon the results of the recently completed first phase of the TINSAL-CVD study. This study compared the effects of 3 different doses of salsalate (3.0, 3.5 and 4.0 grams/day) showing that the 3.5 grams dose had the best efficacy/safety profile. These changes are in Section 1.3, page 5, 5th paragraph; Section 2.3, page 53, 4th paragraph; Section 2.3.4, page 56, 6th paragraph; Section 2.3.5, page 57, 3rd paragraph

Version 1.7:
Treatment with Salsalate (Disalcid™) two grams twice daily.

Version 1.8:
Treatment with Salsalate (Disalcid™) 3.5 grams a day.

Version 1.7:
Subjects will be randomly assigned to receive salsalate 4.0 g/day administered as 500 mg, 4 tablets per oral 2 times a day, or placebo, for 30 months.

Version 1.8:
Subjects will be randomly assigned to receive salsalate 3.5 g/day administered as 500 mg tablets, divided into 2 doses per oral daily, or placebo, for 30 months.

In the inclusion criteria a lower BMI cutoff of 24.5 will be used for patients from Asian origin. This is based on the World Health Organization report (Lancet. 2004 Jan 10; 363(9403):157-63) suggesting that the BMI range of 25-29.9 defining overweight in Caucasians should be adjusted to 23-27.5 in Asians. Therefore, the BMI cutoff of 27 for Caucasians corresponds to approximately 24.5 in the Asians modified scale. This change is in Section 2.1, page 46, 8th paragraph and Section 4.1, page 63, 5th paragraph

Version 1.7:
b) have BMI \( \geq 27 \) kg/m\(^2\) and \( \leq 35 \) kg/m\(^2\) if females and \( \leq 40 \) kg/m\(^2\) if males

Version 1.8:
b) have BMI \( \geq 27 \) kg/m\(^2\) and \( \leq 35 \) kg/m\(^2\) if females and \( \leq 40 \) kg/m\(^2\) if males (to prevent under-representation of minority persons in our investigations, we will use a BMI \( \geq 24.5 \) for subjects from Asian origin

Version 1.7:
b) BMI \( \geq 27 \) kg/m\(^2\) and \( \leq 35 \) kg/m\(^2\) if female and \( \leq 40 \) kg/m\(^2\) if male

Version 1.8:
b) BMI \( \geq 27 \) kg/m\(^2\) and \( \leq 35 \) kg/m\(^2\) if female and \( \leq 40 \) kg/m\(^2\) if male (a BMI \( \geq 24.5 \) for subjects from Asian origin

Exclusion criteria number 3 is now clarified in section 4.2 page 64:

Version 1.7:
3. \( \geq 70\% \) occlusion on MDCTA

Version 1.8:
3. significant obstructive disease (\( >70\% \)) in left main coronary artery, ostial LAD or three-vessel disease by MDCTA

In agreement with this change we made the following change in section 2.2.2, page 51, 6th paragraph and section 2.3.3, page 55, 2nd paragraph:
Version 1.7:

**General Safety:** Subjects with a suspect critical lesion (≥ 70%) on MDCTA will have these results immediately transmitted to their cardiologist and be referred for ETT and/or catheterization, if indicated, via referring cardiologist. Those with ≥ 70% occlusion will be excluded. They can then be reevaluated for inclusion/exclusion criteria after appropriate intervention if within 3 months of MDCTA.

Version 1.8:

**General Safety:** Subjects with a suspect critical lesion (significant obstructive disease of ≥ 70% in left main coronary artery, ostial LAD or three-vessel disease) on MDCTA will have these results immediately transmitted to their cardiologist and be referred for ETT and/or catheterization, if indicated, via referring cardiologist. Those with significant obstructive disease in left main coronary artery, ostial LAD or three-vessel disease will be excluded. They can then be reevaluated for inclusion/exclusion criteria after appropriate intervention if within 3 months of MDCTA.

And also the following change in section 5.1, page 66, 2nd paragraph and section 2.3.3, page 55, 2nd paragraph:

**Version 1.7:**
However, patients may be found to have a coronary lesion of >70% in a proximal vessel that would be considered of very high risk and lead to medical intervention averting a serious and potentially life-threatening event.

**Version 1.8:**
However, patients may be found to have a significant coronary lesion of >70% in a proximal vessel that would be considered of very high risk and lead to medical intervention averting a serious and potentially life-threatening event.

The Exercise Tolerance Test should be performed before visit 2 rather than before visit 1. This was changed in section 2.2.1, page 49, last paragraph; section 5.2.1, page 66, 4th paragraph and section 6.2.4.1, page 74, 2nd paragraph.

In section 4.1, page 83, we deleted a sentence that was an error:

**Version 1.7:**
However, increased abdominal girth and two additional components are necessary for entry to ensure enrollment of subjects with central obesity. Furthermore, as we recognize the importance of the metabolic syndrome in minority populations, and to prevent under-representation of minority persons in our investigations, we will use developed ethnic specific waist ratios.

**Version 1.8:**
However, as we recognize the importance of the metabolic syndrome in minority populations, and to prevent under-representation of minority persons in our investigations, we will use developed ethnic specific waist ratios.

As all patients will have to have a recent ETT done before entering the study, we deleted the requirements to have their cardiologist approve their participation in the study. These changes were made in section 2.2, page 50, 1st paragraph; section 5.2.1, page 66, 4th paragraph and section 6.2.1.4, page 74, 2nd paragraph.
Protocol Amendment: Version 1.7

Section 2.3.4, page 56: a paragraph specifying recommendations for study subjects' care providers was added.

Version 1.7

All study subjects will be recommended to continue follow-up with their clinical cardiologist every 6 months. We will recommend all providers participating in the study to follow standard of care treatments for participants with heart disease, in accordance with the guidelines of the American College of Cardiology, American Heart Association, and American Diabetes Association guidelines for treatment of patients with coronary heart disease (encouraged to follow good medical practice guidelines).

A section regarding Data Protection and safety was added on page 69:

Version 1.7

5.2.7 Data Protection and safety:

All study staff will be trained and certified in privacy protection, the Health Insurance Portability and Accountability Act (HIPAA), and the ethical conduct of clinical research. We cannot fully protect patient confidentiality in this study as patient registration at the Beth Israel Deaconess Medical Center (BIDMC), radiology procedures, and safety laboratories at Quest Diagnostic Laboratories are all linked to patient name. However, persons working in BIDMC registration, radiology department, as well as other professionals who may come in contact with identifiable information are professionally trained to respect this sensitive information. The same holds true for professionals at Quest Diagnostics, which operates under a Massachusetts State License. Identifiable patient information, i.e. patient name, will also be used to communicate with the patient/subject's cardiologist. However, the site provider(s) is/are also aware of the private information. Use of identifiable information is essential to facilitate communication with study personnel directly involved in patient follow-up, especially as sites use different medical record numbers than either the BIDMC or the study specific ID.

The results from Quest Diagnostic Laboratories will be transferred through the secure, protected data stream existing between Quest and the BIDMC which is used and approved for the transfer of clinical information. The medical and laboratory data will be maintained through our data coordinating center such that individual data will be available only to the steering committee, medical monitor, and data coordinating center, and providers directly caring for the individual study subject using a password protected system.

Results of the MDCTA will likewise be stored through the trial specific secure electronic medical data system with access restricted to the steering committee members, the medical monitor, the data coordinating staff, and the study staff who are directly involved in the care of the individual patient using the same password protection system.

Names, social security numbers and other types of personal identification will not appear on study forms, data files or materials sent to anyone outside the approved local study site staff. All study subjects’ data and specimens shared with other bodies (including the research core laboratory) will be identified only by their coded study ID with no personal identifiers. The link between blood samples and the individual that it belongs to will be available only to the individual care provider, the steering committee and the data coordinating center.

Access to the study website will be granted only to pre-authorized study staff using a stringent personal password. Each site will have access only to their site patients’ information. Solely the steering committee, the study medical monitor and the data coordinating center will have access to all study patients information. Individual identifiers will be removed from records prior to data
assembly and statistical analysis, and only the code ID’s will be used. No subject will be identified in any publication from this study.

All study forms and materials collected for the study are stored in secure, locked locations. Each study site will maintain a file on each patient that includes personal identifiers, including contact information linked to the participant coded study ID number. These data are not transferred to the Administrative Core. The files are kept in secure locations and the study site is responsible for taking every other reasonable measure (those set by the state, the site, and the study) to ensure and maintain record confidentiality and patient privacy.
Protocol Amendment: Version 1.6

The wording “Omacor (now known as Lovaza)” was removed throughout the protocol. Instead we defined “Lovaza (previously known as Omacor)” in the beginning (section 1.3, page 5 3rd paragraph) and then left only Lovaza in the rest of the protocol (pages 5, 44, 48, 50, 72 and 74).

Section 2.2, page 48, last paragraph: we clarified that Take Control Promise margarine will not be provided from Unilever, but that subjects will be recommended to use Promise margarine.

Version 1.5
Study subjects in the active lifestyle group will be prescribed four Omacor (now known as Lovaza) (omega 3) capsules, which will be shipped directly to study subjects from Pharmacy Benefits Management (PBM) Plus, Inc. and two serving per day of full fat high alpha linolenic acid Promise Take Control margarine from Unilever (which also contains quite a bit of linoleic acid and plant sterols-and will ensure adequate intakes of essential fatty acids).

Version 1.6
Study subjects in the active lifestyle group will be prescribed four Lovaza (omega 3) capsules, which will be shipped directly to study subjects from Pharmacy Benefits Management (PBM) Plus, Inc. Subjects will be recommended to use two servings (2 tablespoons) per day of full fat high alpha linolenic acid Promise margarine from Unilever (which also contains quite a bit of linoleic acid and plant sterols-and will ensure adequate intakes of essential fatty acids).

Section 5.2.4, end of page 67 and beginning of page 68 and section 6.2.1.4, page 75, 3rd paragraph: we clarified that Metoprolol will be given orally before MDCTA rather than intravenously, and IV metoprolol will be added only if heart bit remains above 65.

Version 1.5
For subjects with a heart rate greater than this, the beta-blocker Metoprolol will be administered to reduce the rate to approximately 60 beats per minute. The Metoprolol dose (5 mg every 5 minutes for no more than 3 times or until subject’s heart rate goes down to 60 beats per minute) will be administered in the presence of a physician, through an intravenous injection one hour before the scan. Intravenous Metoprolol can cause bradycardia, heart rate below 60 beats per minute and hypotension however, the maximum dose of Metoprolol (15 mg) proposed is not associated with light-headedness, severe slowing of the heart, nausea or vomiting. Continuous use in the treatment dose range may result in these symptoms and/or dizziness in 10% of patients; mild depression in 5%; and lowering or severe slowing of heart rate in 3%.

Version 1.6
For subjects with a heart rate greater than this, the beta-blocker Metoprolol (50 mg) will be administered by mouth to reduce the rate to approximately 60 beats per minute one hour before the MDCTA. If the subject’s heart rate remains greater than 65 beats per minute at 1 hour after 50 mg of metoprolol is given by mouth, 5 mg metoprolol will be given every 5 minutes up to 3 times or until the heart rate goes down to 65 beats per minute. This will be given through an intravenous injection in the presence of a physician. Metoprolol can cause bradycardia (or slow heart rate) which could also reduce blood pressure. However, this dose of Metoprolol is not usually associated with light-headedness, severe slowing of the heart, nausea or vomiting.
Protocol Amendment: Version 1.5

Section 4.1, page 63, 1st paragraph: Based on the recently published COURAGE trial (NEJM 2007; 356:1503-16) we further clarified our inclusion/exclusion criteria:

Version 1.4
Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction, stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery, abnormal exercise tolerance test or an area of reversible ischemia on nuclear imaging, with subsequent revascularization.

Version 1.5
Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction, stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery, or abnormal exercise tolerance test or an area of reversible ischemia on nuclear imaging study or pharmacologic stress, with subsequent revascularization, or angioplasty, or abnormal exercise treadmill stress test with or without nuclear imaging or echocardiography with the following exclusions:

Exclusions based on nuclear imaging:
1. Transient cavity dilation
2. More than one vascular territory involved with reversible defect (multiple defects)
3. Reversible defects involving the anterior wall, septum or apex (LAD territory)

Exclusions based on echocardiography imaging:
1. More than one vascular territory involved with inducible wall motion abnormalities (multiple defects)
2. Inducible wall motion abnormalities involving the anterior wall, septum or apex (LAD territory)

Section 2.1, page 45, 3rd paragraph

Version 1.4
Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction, stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery, abnormal exercise tolerance test or an area of reversible ischemia on nuclear imaging with subsequent revascularization,

Version 1.5
Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction, stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery or abnormal exercise tolerance test.

Section 2.2.1, page 49, 2nd paragraph, Section 5.2.1, page 66, 4th paragraph and Section 6.2.1.4, page 73, 2nd paragraph we have added:
For patients without angina during stress and with a reversible defect on nuclear imaging or an inducible wall motion abnormality during echocardiography without subsequent revascularization, exercise will be limited to 70% of the peak HR achieved during exercise.

Section 5.2.4, page 68 and section 6.2.1.4, page 74 a paragraph about potential adverse reactions of nitroglycerin was added:

Version 1.5
Nitroglycerin: Sublingual nitroglycerin (0.4 mg) will be given to make the coronary arteries more prominent on MDCTA. This is standard procedure during MDCTA. Rarely, this causes a
headache. The use of medications to improve erectile dysfunction (Viagra, Levitra, Cialis) in conjunction with nitroglycerin can cause severe drop in blood pressure and shock. Therefore subjects will be instructed not take these medications for 72 hours prior to the MDCTA testing. Subjects with known allergy to nitroglycerin will not be able to participate in the study.

We have modified our inclusion criteria to include subjects who have not tolerated statin therapy. These changes are noted on pages 45, 46, 61 and 63 of the protocol:

Section 2.1 page 45, 4th paragraph

Version 1.4
Subjects who have had coronary artery bypass grafting or unstable angina are not eligible. Subjects must be on a statin.

Version 1.5
Subjects who have had coronary artery bypass grafting or unstable angina are not eligible. Subjects must be on a statin or unable to tolerate a statin.

and

Section 2.1 page 45, 7th paragraph and section 4.1 page 63 4th paragraph

Version 1.4
c) on a stable dose of an HMG CoA reductase inhibitor (statin) for 1 month at screening

Version 1.5
c) on a stable dose of an HMG CoA reductase inhibitor (statin) for 1 month at screening or unable to tolerate a statin;

and

Section 2.1 page 45, 8th paragraph we deleted:
All patients will be on a statin.

and

Section 2.1 page 46, 6th paragraph:

Version 1.4
CVD patients on statins with metabolic syndrome would be scheduled…

Version 1.5
CVD patients with metabolic syndrome would be scheduled…

and

Section 3.4 page 61, 1st paragraph:

Version 1.4
As all of the patients in this trial will be on statin

Version 1.5
As the majority of the patients in this trial will be on statin

The screening window has been changed from 1 month to 3 months on pages 44, 46, 49, 52 and 55.

The number of lifestyle sessions was changed from 16 to 12 on pages 47 and 48.

Section 4.2 page 65: In the exclusion criteria part we have increased the alcohol consumption upper limit from 10 to 14 units/week on page 65 of the protocol.
The period in which the exercise tolerance test at screening is valid has been increased from 6 to 12 months. See changes on: Section 2.1 page 45, 8th paragraph; Section 2.2.1 page 49, 1st paragraph; Section 4.1 page 63, 3rd paragraph; Section 5.2.1 page 66, 4th paragraph; Section 6.2.1.4 page 73, 2nd paragraph.

In the inclusion criteria we specified that subjects should be at least 6 months post myocardial infarction and/or revascularization procedure:

Section 2.1 page 45, 4th paragraph and Section 4.1 page 63, 4th paragraph:

Version 1.5
Subjects should be at least 6 months after a myocardial infarction and/or revascularization procedure to be eligible.
Protocol Amendment: Version 1.4

Section 1.2, page 5, 1st paragraph: The wordings regarding the primary endpoint were slightly modified as requested by BIDMC IRB:

Version 1.3
The primary endpoint is change in coronary artery plaque volume measured by MDCTA from baseline to 30 months.

Version 1.4
The single primary endpoint is change in total plaque volume in the coronary arteries assessed by MDCTA from baseline to 30 months.

Section 1.3, page 6, 4th paragraph has been modified as requested by BIDMC IRB to specify the CRP is only one of many secondary outcomes in this study:

Version 1.3
2) Reduce inflammation and cardiovascular risk as measured by C-reactive protein.

Version 1.4
1) Reduce inflammation and cardiovascular risk as measured by C-reactive protein, which is only one of many secondary outcomes in this study.

The BIDMC IRB requested to be specific that the label "lifestyle intervention" includes the daily use of Omacor, a prescription medication available in the US only with a physician's prescription. This was added to Section 1.3, page 5, 4th paragraph and page 6, 2nd paragraph; section 2.1, page 45, 2nd paragraph; section 6.2.4.1, page 75, 3rd paragraph.

Section 1.3.13, page 57, 6th paragraph: The BIDMC requested to be specific that CRP is merely one of many secondary endpoints of this study.

Version 1.3
Reductions in the inflammatory marker CRP is...

Version 1.4
Reductions in the inflammatory marker CRP, one of many secondary endpoints in this study, are...

Section 2.1, page 46, 6th paragraph:

Version 1.3
Patients with T2D must have a fasting glucose of ≤ 160 mg/dl at screening

Version 1.4
Patients with T2D must have a fasting glucose of ≤ 200 mg/dl at screening

Section 2.2, page 50, 5th paragraph: as requested by BIDMC the way in which subjects will receive Omacor is no more specified:

Version 1.3
Study subjects in the active lifestyle group also receive four Omacor (omega 3) capsules, from Reliant

Version 1.4
Study subjects in the active lifestyle group will be prescribed four Omacor (omega 3) capsules, which will be shipped directly to study subjects from Pharmacy Benefits Management (PBM) Plus, Inc.
In compliance with the Decision Memorandum for Clinical Trial Policy (CAG-00071R), Date: July 9, 2007, for Medicare service, a paragraph regarding Medicare subjects was added: (http://www.cms.hhs.gov/mcd/viewdecisionmemo.asp?id=186)

**Section 4.1, page 65, 6th paragraph:**

Version 1.4

Subjects with Medicare health insurance will have equal opportunity to participate in this study. Considering their older age, Medicare subjects are more likely to be diagnosed with the metabolic syndrome and coronary heart disease and therefore the results of this study will be especially applicable to the Medicare population.

Reliant, the company that manufactures the omega-3 acid that will be given to the subjects in the lifestyle arm, had changed the brand name of the drug from Omacor to Lovaza. Therefore the phrase “now known as Lovaza” was added to the term Omacor wherever it is mentioned.

In section 1.3, page 4, 4th and 11th paragraphs; section 2.1, page 45 2nd paragraph; section 2.2, page 50, 2nd paragraph; section 2.2.2, page 52, 4th paragraph; section 6.2, page 73, 1st paragraph; and section 6.2.1.4, page 75, 3rd paragraph.

**Section 3.2, page 61, 2nd paragraph:** Randomization stratification by the presence of diabetes:

Version 1.3

The study will use permuted block randomization, stratified by clinical care provider site, to allocate patients to achieve comparability among treatment groups with respect to known and unknown prognostic factors. We plan to stratify only by clinic and adjust any imbalances that may occur across groups on other characteristics using adjustments in the analysis.

Version 1.4

The study will use permuted block randomization, stratified by clinical care provider site, to allocate patients to achieve comparability among treatment groups with respect to known and unknown prognostic factors. In addition we would stratify patients based on the presence of diabetes. As this is a significant cardiac risk factor that is synergistic to cardiac event risk with hypertension and hypercholesterolemia, disproportionate randomization could skew results. We will adjust any imbalances that may occur across groups on other characteristics using adjustments in the analysis.

**Section 2.1 page 47:** The list of participating cardiology sites was removed from the protocol.

**Section 2.1, page 47, 3rd paragraph** the word Ten was changed to Several:

Version 1.3

Ten cardiology groups and the BIDMC cardiology clinic and JDC clinic will participate in the recruitment and the total number of subjects to be consented and screened at each site will be approximately 83 (total 1000) of whom about 67 subjects will qualify and be randomized for the study and will be evaluated by MDCTA (total 800). This permits a 10% MDCTA failure rate, such that 720 subjects will continue participation in the study.

Version 1.4

Several cardiology practice groups and the BIDMC cardiology clinic and JDC clinic will participate in the recruitment.

**Section 4.2, page 66:** exclusion criteria were slightly modified:
Version 1.3
16) Use of continuous oral corticosteroid treatment (more than 2 weeks),

Version 1.4
16) Use of continuous oral corticosteroid treatment (more than 2 weeks), or patients requiring corticosteroids within 3 months

Version 1.3
22) History of peptic ulcer or gastritis

Version 1.4
22) History of peptic ulcer or gastritis within 5 years

Section 2.3.1, page 54, 2nd paragraph: 12 month has been added since the 12 month visit is now at BIDMC/JDC.

Section 2.2.1, page 50, 3rd paragraph and section 5.2.1, page 68, 4th paragraph and section 6.2.4.1, page 75, 2nd paragraph: We have clarified that Dr. Welty will interpret results of ETTs and provide exercise prescriptions:

Version 1.3
The nurse practitioner will review all ETT results and calculate maximal target heart rate. She will review these results with Dr. Welty (cardiologist) who will provide exercise prescriptions for each study subject based on their ETT results.

Version 1.4
Dr. Welty will review all ETT results, calculate maximal target heart rate and provide exercise prescriptions for each study subject based on their ETT results.

Since either a nurse or MD or study coordinator may see subjects we have added the term “research team” or “study coordinator” to “nurse” throughout the protocol.

Section 2.1, page 47, last paragraph:

Version 1.3
However, the nurse at each clinical site will have to fill out information...

Version 1.4
However, the nurse or study coordinator at each clinical site will have to fill out information...

Section 2.1, page 49, 2nd paragraph:

Version 1.3
Once a patient is assigned to a specific study arm, the patient is then followed by that arm’s study nurse and that arm’s principal investigator (PI) for the life of the study.

Version 1.4
Once a patient is assigned to a specific study arm, the patient is then followed by that arm’s study nurse or coordinator and that arm’s principal investigator (PI) for the life of the study.

Section 2.2, page 49, 3rd paragraph:

Version 1.3
This Exercise program will be implemented by the lifestyle managers Dr.’s Francine Welty and Ernst Schaefer, and a study nurse, and nutrition by a study dietitian who will be working under the supervision of Dr. Schaefer to implement the dietary intervention.

Version 1.4
This Exercise program will be implemented by the lifestyle managers Dr.’s Francine Welty and Ernst Schaefer, and a study coordinator, and nutrition by a study dietitian who will be working under the supervision of Dr. Schaefer to implement the dietary intervention.

Section 6.1.1, page 71, 6th paragraph:

Version 1.3
The project leader for lifestyle arm (Welty) and her co-PI Dr. Schaefer and respective study nurses will oversee study patient safety and review the safety and progress of the lifestyle arm on a bi-monthly basis. Dr. Goldfine, will be the responsible principal investigator for salsalate/placebo arm and will oversee study patient safety also bi-monthly. Safety labs will be reviewed as they come in immediately by the nurse for each respective project. Any abnormal labs will then be reviewed immediately with the respective project leader. Reports for any adverse event of moderate or greater severity will be generated by the nurse for each project and reported to the Overall Principal Investigator (Dr. Francine Welty).

Version 1.4
The project leader for lifestyle arm (Welty) and her co-PI Dr. Schaefer and study coordinator will oversee study patient safety and review the safety and progress of the lifestyle arm on a bi-monthly basis. Dr. Goldfine, will be the responsible principal investigator for salsalate/placebo arm and will oversee study patient safety also bi-monthly. Safety labs will be reviewed as they come in immediately by the research team for each respective project. Any abnormal labs will then be reviewed immediately with the respective project leader. Reports for any adverse event of moderate or greater severity will be generated by the research team for each project and reported to the Overall Principal Investigator (Dr. Francine Welty).

Section 6.1.2, page 71, last paragraph:

Version 1.3
The research nurse for each project will ensure collection of data from the clinical sites and review all materials for complete collection.

Version 1.4
The study coordinator or research nurse for each project will ensure collection of data from the clinical sites and review all materials for complete collection.

Section 6.1.2, page 72, 1st paragraph:

Version 1.3
The study nurse would conduct a review of 100% of the informed consents…

Version 1.4
The study coordinator or research nurse would conduct a review of 100% of the informed consents…

Section 6.1.7, page 72, 6th paragraph:

Version 1.3
The two arms study nurses will review all laboratory reports within 24 hours of completion by the laboratory (Quest Diagnostics).

Version 1.4
The research team will review all laboratory reports within 24 hours of completion by the laboratory (Quest Diagnostics).
Protocol Amendment: Version 1.3

Due to the NHLBI response in review of TINSAL-CVD protocol v1.2 to not include CRP as a primary end-point, CRP was deleted from all the sections discussing co-primary endpoints and added to the secondary end-point sections. The power calculation and statistical analysis sections were modified to reflect these changes. The list of changes is detailed below:

Section 1.1, page 4, 6th and 7th paragraphs:

Version 1.2
(1) Since Western diet and obesity activate the NF-κB cascade, we hypothesize that weight loss achieved through dietary and exercise intervention will promote vascular remodeling and regression of soft plaque and reduce the sub-acute inflammatory process and cardiovascular risk assessed by serum CRP.

(2) We will directly target the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long-term use in humans. We hypothesize that IKKβ/NF-κB inhibition via salicylate intervention will promote vascular remodeling and regression of soft plaque and reduce the sub-acute inflammatory process and cardiovascular risk assessed by serum CRP.

Version 1.3
(1) Since Western diet and obesity activate the NF-κB cascade, we hypothesize that weight loss achieved through dietary and exercise intervention will suppress the sub-acute inflammatory process to promote vascular remodeling and regression of soft plaque.

(2) We will directly target the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long-term use in humans. We hypothesize that IKKβ/NF-κB inhibition via salicylate intervention will suppress the sub-acute inflammatory process to promote vascular remodeling and regression of soft plaque.

Section 1.2, page 5, 1st paragraph:

Version 1.2
The primary endpoints are changes in coronary artery plaque volume measured by MDCTA and serum CRP from baseline to 30 months.

Version 1.3
The primary endpoint is changes in coronary artery plaque volume measured by MDCTA from baseline to 30 months.

Section 1.3, page 5, 4th paragraph:

Version 1.2
The effect on sub-acute inflammation by each intervention will be assessed using serum levels of CRP and Coronary plaque remodeling will be assessed by MDCTA.

Version 1.3
Coronary plaque remodeling by each intervention will be assessed by MDCTA.

Section 1.3, page 5, 5th paragraph:

Version 1.2
Hypothesis: Since Western diet and obesity activate the NF-κB cascade in rodents, we hypothesize that weight loss achieved through dietary and exercise intervention or directly targeting the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using
a salicylate that has been proven to be safe for long term use in humans, will suppress sub-acute inflammatory process to promote vascular remodeling and regression of soft plaque assessed by MDCTA and reduce serum levels of CRP, in patients with stable coronary artery disease and the metabolic syndrome.

Version 1.3
Hypothesis: Since Western diet and obesity activate the NF-κB cascade in rodents, we hypothesize that weight loss achieved through dietary and exercise intervention or directly targeting the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long term use in humans, will suppress sub-acute inflammatory process to promote vascular remodeling and regression of soft plaque assessed by MDCTA, in patients with stable coronary artery disease and the metabolic syndrome.

Section 1.3, page 5, 6th paragraph:

Version 1.2
Coronary plaque and serum CRP will be measured before and following a 30 month intervention in 3 arms:

Version 1.3
Coronary plaque will be measured before and following a 30 month intervention in 3 arms:

Section 1.3, page 6, 1st paragraph: (Change of order)

Version 1.2
1) Reduce inflammation and cardiovascular risk as measured by C-reactive protein.
2) Promote vascular remodeling as assessed by regression or lack of progression of soft plaque measured by MDCTA.

Version 1.3
1) Promote vascular remodeling as assessed by regression or lack of progression of soft plaque measured by MDCTA.
2) Reduce inflammation and cardiovascular risk as measured by C-reactive protein.

Section 2.3.5, page 57, 6th paragraph:

Version 1.2
Primary Outcome: The primary outcomes will measure change in soft plaque measured by Computed Tomography (CT) angiography and inflammation measured by C-reactive protein.

Version 1.3
Primary Outcome: The primary outcomes will measure change in soft plaque measured by Computed Tomography (CT) angiography.

Section 3.3, page 61, 4th paragraph:

Version 1.2
This study will have 2 primary outcomes: the change in soft plaque measured by Computed Tomography (CT) angiography and the reduction in inflammation and cardiovascular risk, as measured by CRP between baseline and 30 months.

Version 1.3
The primary outcome will measure change in soft plaque measured by Computed Tomography (CT) angiography between baseline and 30 months.
Section 3.4, page 62 was changed to reflect the changes in primary endpoint on power calculation:

Version 1.2
The effects of lifestyle modification and salsalate therapy on coronary atherosclerosis can be assessed with either markers of inflammation such as CRP or by measuring coronary artery plaque volume using coronary CTA. While changes in CRP will reflect changes in the inflammatory milieu that are strongly associated with adverse events related to unstable plaque, coronary CTA directly measures the plaque volume that may independently assess plaque stability. Because the effects of these interventions on these important measures of coronary atherosclerosis are uncertain, this trial will assess both as independent primary endpoints. A two-sided p-value of 0.025 will be used to evaluate each endpoint to preserve a total type I error of 0.05.

The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. This preliminary data showed a 9.3% decrease in LDL cholesterol. As all of the patients in this trial will be on statin therapy, we expect a 25% smaller treatment effect, resulting in a reduction in LDL cholesterol of 7.0%. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24 ±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume, this trial expects to observe a 4.2% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group, the number of subjects required to have a type II error of 0.2 (80% power) with a p-value of 0.025 is 184 per treatment arm. With an initial randomization of 240 subjects to each arm with a 20% dropout rate, this study will have a power of 0.82.

The power of this study to detect a change in CRP is estimated based on the preliminary data from 37 patients with diabetes mellitus or obesity randomized to receive salsalate (4 g/day) or placebo. The mean reduction in CRP after one month was 1.4 ± 2.1 in the active treatment group compared to 0.6 ± 2.6 in the placebo group. This trial expects to observe similar changes in CRP. The number of subjects required to have a type II error of 0.2 (80% power) with a p-value of 0.025 is 173 per treatment arm. With an initial randomization of 240 subjects to each arm with a 20% dropout rate, this study will have a power of 0.85.

Version 1.3
The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. This preliminary data showed a 9.3% decrease in LDL cholesterol. As all of the patients in this trial will be on statin therapy, we expect a 30% smaller treatment effect, resulting in a reduction in LDL cholesterol of 6.5. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24 ±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume, this trial expects to observe a 4.2% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group, the number of subjects required to have a type II error of 0.2 (80% power) with a p-value of 0.05 is 176 per treatment arm. With an initial randomization of 240 subjects to each arm and a 20% dropout rate, resulting in a total of 192 subjects with assessment of the primary endpoint, this study will have a power of 0.83.

Section 3.5, page 63, 2nd paragraph: CRP was added to the list of secondary endpoints

Version 1.2
2) Reduction of mediators of inflammation in the circulation in addition to CRP, including PAI-1, serum amyloid A, MMP-9 and fibrinogen, pro-inflammatory cytokines including IL-6, TNF-a and IL-1b, the adhesion molecules VCAM-1 and ICAM-1, increase in adiponectin and reduction in serum nitrotyrosine as a marker of oxidative stress.

Version 1.3

1) Reduction of mediators of inflammation in the circulation including CRP, PAI-1, serum amyloid A, MMP-9 and fibrinogen, pro-inflammatory cytokines including IL-6, TNF-a and IL-1b, the adhesion molecules VCAM-1 and ICAM-1, increase in adiponectin and reduction in serum nitrotyrosine as a marker of oxidative stress.

Section 3.6, page 63, last paragraph: The statistical analysis plan was modified to reflect only MDCTA as primary endpoint:

Version 1.2

This trial assesses the change in CRP and coronary plaque volume as measured by MDCTA in three treatment groups. The primary analytic approach will be analysis of variance (ANOVA) using Dunnett's test to compare the active treatment groups to placebo. Because there are two primary endpoints, each will be assessed using a p-value of 0.025 to adjust for performing two comparisons. The primary analysis will be performed on an intent-to-treat basis amongst those subjects who have had the primary endpoint evaluated (change in CRP and/or coronary plaque volume during the study, requiring 2 measurements for each). For the primary endpoint, there is no easy way to address missing data. It is not possible to carry forward values if there is only a baseline measurement. Therefore, patients who do not return for a second CRP measurement or MDCTA will be treated as missing data. Because missing data may represent informative censoring, we will perform a formal survival analysis of both the intervention and placebo groups to determine that there has been no biased loss to follow-up. Additionally, we will perform a sensitivity analysis imputing conservative values for missing data (e.g. the mean change observed in the control group) and optimistic values (e.g. the mean change observed in the active treatment group for missing data in the active treatment group) to further assess the magnitude of any biased losses. We will then impute a follow-up value based on the mean CRP change observed in the control group (a relatively conservative assumption). For certain secondary outcomes, the last observation will be carried forward.

Version 1.3

This trial assesses the change in coronary plaque volume as measured by MDCTA in three treatment groups. The primary analytic approach will be analysis of variance (ANOVA) using Dunnett’s test to compare the active treatment groups to placebo. Statistical significance will be assessed using a p-value of 0.05. The primary analysis will be performed on an intent-to-treat basis amongst those subjects who have had the primary endpoint evaluated (change in coronary plaque volume during the study, requiring 2 measurements). For the primary endpoint, there is no easy way to address missing data. It is not possible to carry forward values if there is only a baseline measurement. Therefore, patients who do not return for a second MDCTA will be treated as missing data. Because missing data may represent informative censoring, we will perform a formal survival analysis of both the intervention and placebo groups to determine that there has been no biased loss to follow-up. Additionally, we will perform a sensitivity analysis imputing conservative values for missing data (e.g. the mean change observed in the control group) and optimistic values (e.g. mean change observed in the active treatment group for missing data in the active treatment group) to further assess the magnitude of any biased losses. We will then impute a follow-up value based on the mean CRP change observed in the control group (a relatively conservative assumption). For certain secondary outcomes, the last observation will be carried forward.
Given the potential for noncompliance with the lifestyle intervention, we will also perform a pre-specified secondary analysis on a per protocol basis. If the primary intent-to-treat analysis is negative (non-significant), the per-protocol analysis will aid in determining if the results are significantly affected by noncompliance.

Analyses of the secondary outcomes are exploratory and will be performed using multiple methods, including ANOVA, linear regression, logistic regression, longitudinal regression, and time to event analyses.

Biostatistical support will be provided by: Dr. Shiva Gautam and Dr. Thomas Hauser and data management support with Dr. Steve Berry and Griffin Weber M.D., PhD.

**Section 2.2, page 50, 2nd paragraph:** The NHLBI response in review of TINSAL-CVD protocol v1.2 requested that Slimfast would not be part of the lifestyle intervention protocol. Therefore the relevant sentence was deleted:

**Version 1.2**
For the first 6 months, they will drink 1 scoop of Slimfast in skim milk at breakfast and either a Slimfast bar or Slimfast drink at lunch. For the next 6 months of the study they will have 1 serving of Slimfast a day.

**Version 1.3**
Protocol Amendment: Version 1.2

The study would have 2 primary endpoints: changes in plaque volume measured by MDCTA and serum CRP instead of one endpoint. This change is being reflected in all the section detailed below:

Section 1.1, page 4, 6th and 7th paragraphs:

Version 1.1
(1) Since Western diet and obesity activate the NF-κB cascade, we hypothesize that weight loss achieved through dietary and exercise intervention will reduce the sub-acute inflammatory process and cardiovascular risk assessed by serum CRP.

(2) We will directly target the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long-term use in humans. We hypothesize that IKKβ/NF-κB inhibition via salicylate intervention will reduce the sub-acute inflammatory process and cardiovascular risk assessed by serum CRP.

Version 1.2
(1) Since Western diet and obesity activate the NF-κB cascade, we hypothesize that weight loss achieved through dietary and exercise intervention will promote vascular remodeling and regression of soft plaque and reduce the sub-acute inflammatory process and cardiovascular risk assessed by serum CRP.

(2) We will directly target the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long-term use in humans. We hypothesize that IKKβ/NF-κB inhibition via salicylate intervention will promote vascular remodeling and regression of soft plaque and reduce the sub-acute inflammatory process and cardiovascular risk assessed by serum CRP.

Section 1.2, page 5, 1st paragraph:

Version 1.1
The primary endpoint is change in serum CRP from baseline to 30 months.

Version 1.2
The primary endpoints are changes in coronary artery plaque volume measured by MDCTA and serum CRP from baseline to 30 months.

Section 1.3, page 5, 5th paragraph:

Version 1.1
Hypothesis: Since Western diet and obesity activate the NF-κB cascade in rodents, we hypothesize that weight loss achieved through dietary and exercise intervention or directly targeting the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long term use in humans, will suppress sub-acute inflammatory process measured by serum levels of CRP in patients with stable coronary artery disease and the metabolic syndrome.

Version 1.2
Hypothesis: Since Western diet and obesity activate the NF-κB cascade in rodents, we hypothesize that weight loss achieved through dietary and exercise intervention or directly targeting the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long term use in humans, will suppress sub-acute inflammatory process to promote vascular remodeling and regression of soft
plaque assessed by MDCTA and reduce serum levels of CRP, in patients with stable coronary artery disease and the metabolic syndrome.

Section 1.2, page 5, 6th paragraph:

Version 1.1
Serum CRP will be measured before and following a 30 month intervention in 3 arms:

Version 1.2
Coronary plaque and serum CRP will be measured before and following a 30 month intervention in 3 arms:

Section 2.3.5, page 57, 6th paragraph:

Version 1.1
Primary Outcome: The primary outcome will be inflammation measured by C-reactive protein.

Version 1.2
Primary Outcome: The primary outcomes will measure change in soft plaque measured by Computed Tomography (CT) angiography and inflammation measured by C-reactive protein.

Section 3.3, page 61, 4th paragraph:

Version 1.1
The primary outcome will be the reduction in inflammation and cardiovascular risk, as measured by CRP between baseline and 30 months.

Version 1.2
This study will have 2 primary outcomes: the change in soft plaque measured by Computed Tomography (CT) angiography and the reduction in inflammation and cardiovascular risk, as measured by CRP between baseline and 30 months.

Section 3.4, page 62 was changed to reflect the changes in primary endpoint on power calculation:

Version 1.1
The sample size determination is based on the definition of the primary outcome and the method of analysis. The primary outcome is the change in CRP from baseline to the final assessment at 30 months. The primary statistical analysis will compare the change in CRP in each of the treatment groups using analysis of variance (ANOVA) with the Bonferroni correction for multiple comparisons.

The power of this study was estimated using preliminary data from 37 patients with diabetes mellitus or obesity. These patients were randomized to receive salsalate (4 g/day) or placebo. The mean reduction in CRP after one month was $1.4 \pm 2.1$ in the active treatment group compared to $0.6 \pm 2.6$ in the placebo group. With a type I error of 0.05, a type II error of 0.2 (power 0.8), and assuming a 20% dropout rate over the course of the study, an initial sample size of 209 per treatment group is required. With a planned initial enrollment of 240 per treatment group, this study will have a power of 0.85. This calculation assumes relative normality of the data. While the raw CRP measurements are highly skewed, the difference in CRP measurements is not highly skewed and is sufficiently normal that the central limit theorem readily applies.

The sample size determination is based on the definition of the primary outcome and the method of analysis. The primary outcome is the change in CRP from baseline to the final assessment at 30 months. The primary statistical analysis will compare the change in CRP in each of the treatment groups using analysis of variance (ANOVA) with the Bonferroni correction for multiple comparisons.
The effects of lifestyle modification and salsalate therapy on coronary atherosclerosis can be assessed with either markers of inflammation such as CRP or by measuring coronary artery plaque volume using coronary CTA. While changes in CRP will reflect changes in the inflammatory milieu that are strongly associated with adverse events related to unstable plaque, coronary CTA directly measures the plaque volume that may independently assess plaque stability. Because the effects of these interventions on these important measures of coronary atherosclerosis are uncertain, this trial will assess both as independent primary endpoints. A two-sided p-value of 0.025 will be used to evaluate each endpoint to preserve a total type I error of 0.05.

The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. This preliminary data showed a 9.3% decrease in LDL cholesterol. As all of the patients in this trial will be on statin therapy, we expect a 25% smaller treatment effect, resulting in a reduction in LDL cholesterol of 7.0%. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24 ±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume, this trial expects to observe a 4.2% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group, the number of subjects required to have a type II error of 0.2 (80% power) with a p-value of 0.025 is 184 per treatment arm. With an initial randomization of 240 subjects to each arm with a 20% dropout rate, this study will have a power of 0.82.

The power of this study to detect a change in CRP is estimated based on the preliminary data from 37 patients with diabetes mellitus or obesity randomized to receive salsalate (4 g/day) or placebo. The mean reduction in CRP after one month was 1.4 ± 2.1 in the active treatment group compared to 0.6 ± 2.6 in the placebo group. This trial expects to observe similar changes in CRP. The number of subjects required to have a type II error of 0.2 (80% power) with a p-value of 0.025 is 173 per treatment arm. With an initial randomization of 240 subjects to each arm with a 20% dropout rate, this study will have a power of 0.85.

Section 3.6, page 63, last paragraph: The statistical analysis plan was modified to reflect 2 primary endpoints:

Because this trial assesses the change in CRP in three treatment groups, the primary analytic approach will be analysis of variance (ANOVA) with the Bonferroni correction for multiple comparisons. The primary analysis will be performed on an intent-to-treat basis amongst those subjects who have had the primary endpoint evaluated (change in CRP during the study, requiring 2 measurements). For the primary endpoint, there is no easy way to address missing data. It is not possible to carry forward values if there is only a baseline CRP. Therefore, patients who do not return for a second CRP measurement will be treated as missing data. Because missing data may represent informative censoring, we will perform a formal survival analysis of both the intervention and placebo groups to determine that there has been no biased loss to follow-up. Additionally, we will perform a sensitivity analysis imputing conservative values for missing data (e.g. the mean CRP change observed in the control group) and optimistic values (e.g. mean CRP change observed in the active treatment group for missing data in the active treatment group) to further assess the magnitude of any biased losses. We will then impute a follow-up value based on the mean CRP change observed in the control group (a relatively conservative assumption). For certain secondary outcomes, the last observation will be carried forward.
This trial assesses the change in CRP and coronary plaque volume as measured by MDCTA in three treatment groups. The primary analytic approach will be analysis of variance (ANOVA) using Dunnett’s test to compare the active treatment groups to placebo. Because there are two primary endpoints, each will be assessed using a p-value of 0.025 to adjust for performing two comparisons. The primary analysis will be performed on an intent-to-treat basis amongst those subjects who have had the primary endpoint evaluated (change in CRP and/or coronary plaque volume during the study, requiring 2 measurements for each). For the primary endpoint, there is no easy way to address missing data. It is not possible to carry forward values if there is only a baseline measurement. Therefore, patients who do not return for a second CRP measurement or MDCTA will be treated as missing data. Because missing data may represent informative censoring, we will perform a formal survival analysis of both the intervention and placebo groups to determine that there has been no biased loss to follow-up. Additionally, we will perform a sensitivity analysis imputing conservative values for missing data (e.g. the mean change observed in the control group) and optimistic values (e.g. the mean change observed in the active treatment group for missing data in the active treatment group) to further assess the magnitude of any biased losses. We will then impute a follow-up value based on the mean CRP change observed in the control group (a relatively conservative assumption). For certain secondary outcomes, the last observation will be carried forward.

**Section 3.5, page 62, list of secondary outcomes:**

In version 1.2 plaque remodeling by MDCTA was deleted:

**Section 4.1, page 65, 3rd paragraph** - The definitions used to satisfy the criteria defining the Metabolic Syndrome were change to include both subjects with low HDL or subjects treated for low HDL:

- **Version 1.1**
  HDL-C < 40 mg/dL in men and < 50 mg/dL in women

- **Version 1.2**
  HDL-C < 40 mg/dL in men and < 50 mg/dL in women or on treatment for low HDL

**Section 4.1, page 65, 3rd paragraph** - The definitions used to satisfy the criteria defining the Metabolic Syndrome were change to include both subjects with high triglycerides or subjects treated for high triglycerides:

- **Version 1.1**
  fasting triglyceride > 150 mg/dL (1.69 mmol/L) or triglycerides > 125 mg/dl (1.40 mmol/L) on concomitant statin therapy

- **Version 1.2**
  fasting triglyceride > 150 mg/dL (1.69 mmol/L) or on treatment for high triglycerides

**Section 4.1, page 65, 4th paragraph** - The inclusionary cutoff level for fasting glucose above which subjects with T2D cannot be included in the study was increased from 160 to 200 in order not lose potential study subjects because of coincidental one time high glucose:

- **Version 1.1**
  Patients with T2D must have a fasting glucose of ≤ 160 mg/dl at screening

- **Version 1.2**
  Patients with T2D must have a fasting glucose of ≤ 200 mg/dl at screening

**Section 4.1, page 66, number 5** - The exclusion criteria atrial fibrillation is now more precisely defined as current atrial fibrillation:
5. atrial fibrillation or Wolf-Parkinson-White (WPW) syndrome

Section 4.1, page 67, number 5- The exclusion criteria of significant rheumatologic disease or other inflammatory disease was inadvertently not included. As CRP is one of the primary endpoints of this study the occurrence of chronic inflammatory disease may confound this endpoint:

36. History of significant chronic rheumatologic or other chronic inflammatory disease (including foot ulcers)

Section 4.1, page 67, number 34- The exclusion criteria of salsalate use was slightly modified to reflect also use of other NSAID's and to specify that we are excluding people who are taking these drugs on a chronic basis:

Section 5.2.4, page 70, 2nd paragraph:

We revised the acquisition parameters for the liver-fat protocol and by increasing the pitch factor were able to reduce the dose by 2.58 msV, which reduces the Whole Body Dose Equivalent to from 26.78 msV to 24.2 msV, thereby reducing the total body irradiation dose in the study from 5.4 rem to 4.8 rem.
Protocol Amendment: Version 1.1

Section 2.1, Page 47, 6th paragraph- This sentence was modified to reflect collection of blood for creation of cell lines.

Version 1.0
Blood samples will also be obtained and stored for DNA, mononuclear lymphocyte RNA for gene expression, and serum/plasma stored for protein and lipid fraction analysis at a later date, a urine sample will be collected and electrocardiogram performed.

Version 1.1
Among consenting participants, blood samples and immortalized lymphocytes will also be obtained and stored for DNA, mononuclear lymphocyte RNA for gene expression. Serum/plasma will be stored for protein and lipid fraction analysis at a later date, a urine sample will be collected and electrocardiogram performed.

Section 2.2.2, Page 50, last paragraph and section 2.3.3, page 56, 1st paragraph- These sentences was modified to reflect collection of blood for creation of cell lines.

Version 1.0
Blood will be stored for later analysis of inflammatory profiles, DNA and mononuclear lymphocyte RNA.

Version 1.1
Blood will be stored for later analysis of inflammatory profiles, DNA and mononuclear lymphocyte RNA as well as immortalized lymphocytes among consenting participants.
Summary of protocol versions submissions dates:

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* Submitted on BIDMC IRB form B.
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Abbreviations Used

AE    Adverse Event
BIDMC Beth Israel Deaconess Medical Center
BP    Blood Pressure
CVD   Coronary Heart Disease
CoC   Coordinating Center
CRP   C-reactive Protein
DSMB  Data Safety Monitoring Board
eCrCL Estimated Creatinine Clearance
ETT   Exercise tolerance test
FDA   Food and Drug Administration
HFD   High Fat Diet
HOMA-IR Homeostasis Model Assessment of Insulin Resistance
IRB   Institutional Review Board
JDC   Joslin Diabetes Center
MDCTA Multi-Detector Computed Tomographic Angiography
MI    Myocardial Infarction
MOP   Manual of Procedures
NASH  Nonalcoholic Steatohepatitis
NEIRB New England Investigation Review Board
NF-κB Nuclear Factor Kappa B
NHLBI National Heart, Lung, and Blood Institute
NIH   National Institutes of Health
SAE   Serious Adverse Event
T2D   Type 2 Diabetes
WT    Wild Type
CHAPTER 1: INTRODUCTION AND RATIONALE

The National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) has sponsored a collaborative agreement to conduct a clinical treatment trial, entitled Targeting INflammation using SAIsalate or Lifestyle intervention in CardioVascular Disease (TINSAL-CVD). This protocol describes the background, design and organization of the trial.

The protocol was written by the members of the TINSAL-CVD Steering Committee, and approved by the Institutional Review Boards (IRB’s) of each participating clinical center prior to the initiation of recruitment.

The Steering Committee for the TINSAL-CVD Study Group is composed of investigators at Beth Israel Deaconess Medical Center, Joslin Diabetes Center, and Tufts University School of Medicine. Detailed study procedures are provided in the study’s manual of procedures (MOP).

1.1 Specific Aims and Objectives

Inflammation is increasingly accepted as a major contributor to the pathogenesis of CardioVascular Disease (CVD) and type 2 diabetes (T2D). Obesity induced insulin resistance appear to be at the root of the pathogenic process. In this study, we have united important new discoveries in diverse areas of research to determine whether primary targeting of inflammation as a therapeutic goal provides a new route to treat CVD. Diet induced obesity increases chronic sub-acute inflammation. We will target inflammation via lifestyle intervention and by inhibiting the IKKβ/NF-κB pathway, the master regulator of inflammation. We will combine these two interventions with a newly developing, state-of-the-art, minimally invasive imaging technique, multi-detector computed tomographic angiography (MDCTA) that examines dynamic processes in the coronary circulation, including the development and regression of soft and hard plaques. C-reactive protein (CRP) is a marker of inflammation and an established independent risk factor for CVD.

We will use the two different interventions 1) lifestyle and 2) pharmacologic inhibition of IKKβ/NF-κB with the salicylate, salsalate, to target sub-acute inflammation in overweight/obese patients with established CVD and assess the effect of the interventions on regression (or lack of progression) of coronary artery soft plaque assessed by MDCTA. Specific aims therefore include:

(1) Since Western diet and obesity activate the NF-κB cascade, we hypothesize that weight loss achieved through dietary and exercise intervention will suppress the sub-acute inflammatory process to promote vascular remodeling and regression of soft plaque.

(2) We will directly target the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long-term use in humans. We hypothesize that IKKβ/NF-κB inhibition via salicylate intervention will suppress the sub-acute inflammatory process to promote vascular remodeling and regression of soft plaque.

1.2 Overall Design and Study Interventions

The salsalate trial will be a randomized, double-masked, placebo-controlled, clinical trial. In the lifestyle trial, patients will be randomized to lifestyle plus four fish oil capsules daily or current standard of care. The purpose of the study is to compare the effect of lifestyle intervention and salsalate to their respective standard of care and placebo on sub-acute inflammation and coronary plaque, in overweight/obese people with CVD for a period of 30 months. The single primary endpoint is change in total plaque volume in the coronary arteries assessed by MDCTA from baseline to 30 months.
1.3 Background and Significance

Both lifestyle intervention and salsalate are expected to decrease inflammation. However, the proposed mechanisms of action of each intervention are different. Therefore, the specific aims are the same, and are provided at the beginning of this section. Thereafter the background and significance are provided for each intervention separately.

Inflammation is increasingly accepted as a major contributor to the pathogenesis of CVD and T2D. Obesity Induced insulin resistance appear to be at the root of the pathogenic process. We will target inflammation via lifestyle intervention and directly, by inhibiting NF-κB pathway, the master switch of inflammation. We will combine these interventions with a newly developing, state-of-the-art, minimally invasive imaging technique, multi-detector computed tomographic angiography (MDCTA) that examines dynamic processes in the coronary circulation including the development and regression of soft and hard plaques.

Overview: The study is a trial of overweight/obese patients known stable coronary heart disease. There are two separate trials: one with lifestyle modification and Lovaza previously known as Omacor (omega 3 supplementation), a prescription medication available in the US only with a physician’s prescription, and the second trial with IKKβ/NF-κB inhibition using salsalate. Coronary plaque remodeling by each intervention will be assessed by MDCTA. Randomization will occur in a 1:1 ratio of lifestyle to standard of care to salsalate to placebo.

Hypothesis: Since Western diet and obesity activate the NF-κB cascade in rodents, we hypothesize that weight loss achieved through dietary and exercise intervention or directly targeting the inflammatory signaling pathway via inhibition of IKKβ/NF-κB using a salicylate that has been proven to be safe for long term use in humans, will suppress sub-acute inflammatory process to promote vascular remodeling and regression of soft plaque assessed by MDCTA, in patients with stable coronary artery disease and the metabolic syndrome.

Coronary plaque will be measured before and following a 30 month intervention in all patients:

Specific aims will determine whether intensive lifestyle intervention, which includes the daily use of Lovaza, a prescription medication available in the US only with a physician’s prescription compared to usual care, or salsalate treatment, compared to placebo, in overweight/obese patients will:

1) Promote vascular remodeling as assessed by regression or lack of progression of soft plaque measured by MDCTA.
2) Reduce inflammation and cardiovascular risk as measured by C-reactive protein, which is only one of many secondary outcomes in this study.
3) Treat components of the metabolic syndrome assessed by measures of waist circumference, systolic and diastolic blood pressure, lipid profiles (total cholesterol, triglycerides, HDL and LDL), and abdominal adiposity quantitated by computerized tomography.
4) Reduce mediators of inflammation in the circulation in addition to CRP, including PAI-1, serum amyloid A, MMP-9 and fibrinogen, pro-inflammatory cytokines including IL-6, TNF-α and IL-1β, the adhesion molecules VCAM-1 and ICAM-1, increase adiponectin and reduce serum nitrotyrosine as a marker of oxidative stress.
5) Reduce insulin resistance assessed by fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR).
6) Reduce inflammation in the liver associated with nonalcoholic steatohepatitis (NASH), a newly recognized component of the metabolic syndrome, and reduce fatty liver quantitated by computerized tomography and levels of AST and ALT as markers of liver inflammation related to NASH.

Although we are not specifically studying genetics, we will establish and maintain a large clinical/DNA sample database and store specimens to determine how genetic and protein variation influence therapeutic success in a future grant.

1.3.1 Coronary Heart Disease Overview

CVD remains the leading cause of death and disability in our society. CVD is caused by coronary atherosclerosis with significant vascular remodeling of both calcified and non-calcified plaques during this process. The disease is characterized by lipid deposition, foam cell formation and inflammation, smooth muscle cell proliferation, excess collagen formation, calcification, remodeling of the vessel and thrombosis. The soft plaque is lipid rich and appears to be more susceptible to plaque rupture than calcified fibrous plaque, and therefore has been called “vulnerable plaque.” New technology using MDCTA with injection of intravenous contrast material allows for the imaging of soft non-calcified, lipid-rich plaque, as well as calcified plaque in coronary arteries.

Significant CVD risk factors in addition to age and gender include hypertension, smoking, diabetes, increased low density lipoprotein cholesterol (LDL-C) (> 160 mg/dl), and decreased high density lipoprotein cholesterol (HDL-C) (< 40 mg/dl). Emerging risk factors include altered lipoprotein subspecies, increased apolipoprotein B (apoB) and lipoprotein(a), decreased apolipoprotein A-I (apoA-I), elevated levels of free fatty acids, type of plasma fatty acid, insulin, various inflammatory markers such as C reactive protein (CRP), fibrinogen, and serum amyloid A (all made in liver), as well as the cytokines interleukin-1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNFα), adiponectin secreted by adipocytes, and lipoprotein-associated phospholipase A2 (Lp-PLA2) and secretory phospholipase A2, which are synthesized and released by macrophages. Other important biochemical markers include C-peptide, urine albumin and creatinine, plasminogen activator inhibitor (PAI-1), leptin, intracellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), nitrotyrosine (measure of oxidative stress), folate, vitamins B6 and B12, and homocysteine.

Diets rich in calories, saturated fat, cholesterol, and refined carbohydrates in animals and humans lead to excess abdominal obesity and increased fat in the liver, which in turn is related to over-expression of hepatic NF-κB, and elevated levels of plasma free fatty acids, insulin, triglycerides, remnant particles, LDL-C and inflammatory markers and decreased levels of HDL-C. Diets lower in calories and richer in essential fats, along with exercise, as well as drug treatment with statins, fibrates, niacin and PPAR a agonists have all been shown to reduce body weight, central adiposity, lipids, inflammatory markers and CVD risk, and to have a favorable impact on glucose homeostasis. MDCTA can also be used to image and quantitate abdominal and liver fat.

1.3.2 Metabolic Syndrome Overview

In 1988, Reaven observed that obesity, insulin resistance, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia and dyslipidemia commonly occur together. Originally termed Syndrome X, this constellation of metabolic abnormalities is now known as the metabolic syndrome and appears to have insulin resistance as a central characteristic. Although definitions remain controversial, one adopted definition is that of the ATP III, which defines metabolic syndrome as the presence of any 3 of 5 clinical diagnostic traits: 1) abdominal girth > 35 inches (88 cm) in women, > 40 inches (102 cm) in men; 2) HDL-C < 40 mg/dL in men.
and < 50 mg/dL in women; 3) fasting triglyceride > 150 mg/dL (1.69 mmol/L); 4) blood pressure \(\geq 130/85\) mm Hg; and 5) fasting glucose \(\geq 110\) mg/dL (>6.1 mmol/L) \[5\]. For consistency, we have adopted this definition, with the newer established modifications to the abdominal girth limits based on ethnicity and the newer criterion of glucose \(\geq 100\) mg/dL (>5.6 mmol/L).

Each of the metabolic factors within the metabolic syndrome is itself an established risk factor for atherosclerosis and CVD, and indeed, CVD is the major adverse consequence of metabolic syndrome. In parallel, obesity/overweight and insulin resistance also increase the risk of T2D, which in turn is associated with an increased risk of CVD. As the prevalence of obesity has exploded, so has the prevalence of T2D, the metabolic syndrome and CVD. We have compelling data that generates a hypothesis to link obesity via inflammation to the systemic metabolic consequences including insulin resistance, T2D and CVD.

Pathogenesis of Metabolic Syndrome

Insulin Resistance and a Pro-inflammatory State

Growing evidence over recent years supports a potential role for cytokine-associated, sub-acute inflammation in the pathogenesis of insulin resistance and T2D. Inasmuch as insulin resistance is an underlying feature of the metabolic syndrome, obesity- and Western diet-induced inflammation may also play a role in this constellation of abnormalities associated with heightened risk of atherosclerosis. Atherosclerotic cardiovascular disease is itself increasingly thought to be a disease of chronic sub acute inflammation. These interrelationships suggest that inflammation may be the basis of a “common soil” involved in the pathogenesis of both T2D and atherosclerosis.

Elevations in components of the acute-phase response and inflammation more generally have been convincingly shown to accompany CVD and T2D. Elevated markers common to both disorders include PAI-1, CRP, fibrinogen, IL-6 and IL-1\(\beta\). CRP has been shown to be a significant independent marker of CVD and T2D risk \[6-9\]. However, one big question in the field has been whether these are simply correlative markers for the process or causatively involved in their pathogenesis.

Proinflammatory cytokines, including IL-1\(\beta\), IL-6 and TNF-\(\alpha\), are produced by immune and inflammatory cells, such as macrophages and monocytes, and by adipose tissue and liver, particularly in response to over-nutrition. The cytokines IL-1\(\beta\), IL-6 and TNF-\(\alpha\) also act on liver to produce a characteristic dyslipidemia associated with T2D-increased VLDL and decreased HDL. In addition, these cytokines promote the release of acute-phase proteins, which are atherosclerotic risk factors such as fibrinogen. Circulating cytokines may act directly on the endothelium to promote plaque formation.

NF-\(\kappa\)B is the master regulator of over 200 genes involved in innate immunity, inflammation and apoptosis. As a central mediator of inflammation, NF-\(\kappa\)B orchestrates the synthesis of many of the established mediators of plaque formation in the vasculature, including pro-inflammatory cytokines (e.g. TNF-\(\alpha\), IL-6, IL-1\(\beta\)), macrophage recruiting factors (e.g. MCP-1 and the MIPs), surface proteins (e.g. ICAM, VCAM, and E- and P-selectin), remodeling proteases (e.g. MMP2 and MMP9), prothrombotic proteins (PAI-1, fibronectin) and enzymes that promote oxidative stress (e.g. p47phox) as well as many other proteins implicated in the atherosclerotic process (e.g. CRP, SAA1, iNOS).

Central Obesity, Inflammation and Interference with Insulin Signaling

Central obesity has been shown some time ago to lead to an increased production of TNF-\(\alpha\) in adipose tissue. Moreover, plasma levels of pro-inflammatory mediators, TNF-\(\alpha\), IL-6, CRP, MIF and others are increased and expressed by adipose tissue in obese human subjects.
Furthermore, adipose tissue macrophages and blood monocytes in obese subjects also secrete pro-inflammatory cytokines and have increased binding of NF-κB, the key pro-inflammatory transcription factor. Adiponectin is anti-inflammatory and secreted by adipocytes in non-obese subjects. In overweight and obese subjects, adiponectin concentration declines.

Inflammatory mechanisms interfere with insulin signal transduction. TNF-α has been shown to cause insulin resistance by interfering with insulin signal transduction as follows. TNF-α induces serine phosphorylation of IRS-1, which in turn causes serine phosphorylation of the insulin receptor and thus interferes with insulin signal transduction and causes insulin resistance. IL-6 and TNF-α also induce SOCS-3, a protein which causes ubiquitination and proteosomal degradation of IRS-1 and thus also interferes with normal tyrosine phosphorylation of the insulin receptor and IRS-1. This process also reduces the activation of Akt (protein kinase B), a protein which effects the translocation of the insulin-responsive glucose transporter, Glut-4, to the plasma membrane. In the absence of Akt2, there is greater formation of NaDPH oxidase and increased superoxide generation and oxidative stress. Akt-2-null mice develop insulin resistance, mild hyperglycemia and hyperinsulinemia.

While this view is becoming increasingly accepted, the field continues to evolve. Recently we have shown that NF-κB in fat and liver is activated in obesity by fat mass expansion in adipose tissue and liver (steatosis). This sub-acute ‘inflammatory’ process leads to increased local production of various NF-κB targets, including such markers and potential mediators of inflammation as IL-6, TNF-α, IL-1β, MCP-1, A20, PAI-1 and CRP. The cytokines work locally in liver and fat to induce insulin resistance in these tissues. This is evidenced by the Ser/Thr phosphorylation of IRS proteins and the induction of cytokine targets such as the SOCS proteins, both of which are known causes of insulin resistance.

Markers and potential mediators of inflammation are secreted into the circulation. These are produced in fat and liver by adipocytes and hepatocytes as well as macrophages (and Kupffer cells) and other inflammatory cells. This leads to heightened systemic levels of markers and potential mediators of inflammation. We have documented that fat and liver produce cytokines including IL-6, IL-1b, TNF-a and resistin that are induced by obesity and Western diet and lead to systemic insulin resistance. Muscle represents a major contributor to systemic insulin resistance, where NF-κB is not activated but cytokine-mediated pathways are activated in response to inflammation initiated in fat and liver.
Macronutrient intake can be pro-inflammatory itself and induce oxidative stress as shown in Figure 2. A 75-g glucose challenge increased p47phox expression and increased leukocyte superoxide production by 140%. Cream (fat) intake also increased oxidative stress to a similar amount as with the glucose load. Glucose intake also increased intra-nuclear NF-κB binding, decreased IkB expression, increased IKKα and IKKβ (the 2 kinases that phosphorylate IkBα and IkBβ leading to ubiquitination and proteosomal degradation) and also increased the pro-inflammatory transcription factors, AP-1 (thereby increasing MMP 2 and 9) and Egr-1 (increasing TF and PAI-1). A 900 kcal meal high in both glucose and fat from a fast food restaurant also induced NF-κB, reduced IkBα, increased IKKα and IKKβ and increased superoxide radical generation by mononuclear cells. In contrast, a 900-kcal AHA step 2 diet meal rich in fruit and fiber did not increase oxidative stress or inflammation. The concentrations of these gene products, which are increased by acute nutritional intake, are all elevated in the basal state of obese subjects. Intake of a low calorie diet (1000 kcal/d for 4 weeks) reduced both oxidative stress and inflammatory mediators in obese subjects. Moreover, a 48-hour fast in normal subjects reduced the expression of p47phox and lowered ROS more than 50%. Superoxide radicals generated during oxidative stress also activate NF-κB and AP-1. Therefore, it appears that both obesity and macronutrient intake of high fat and/or high glucose foods are pro-inflammatory conditions, which lead to insulin resistance.

We propose that obesity and Western diet stimulate the production of a similar collection of circulating mediators to establish a systemic inflammatory milieu that also influences the vasculature. We hypothesize that heightened systemic inflammatory ‘tone’ increases the risk of atherosclerosis, as it does for insulin resistance. We propose to test whether plaque progression is modified by lifestyle modification, lipid-lowering/HDL-raising, or direct inhibition of NF-κB and the accompanying inflammatory processes.
1.3.3 Background Specific to Lifestyle intervention:

1.3.3.1 Diet and CVD:

Population Studies
Data from the 20 Countries Study (table 1) clearly indicates that intake of butter, dairy products, eggs, meat and poultry and sugar and syrup all have deleterious effects on risk of CVD mortality while grains, fruits, and starchy and non-starchy vegetables decrease the risk of death from CVD [2]. These data are consistent with many other population studies that report such relationships. Moreover, countries with the highest intakes of saturated fat, such as Russia and the countries of eastern Europe, have the highest rates of age adjusted CVD mortality rates in the world, while the Mediterranean countries and especially Japan, have the lowest saturated fat intakes and the lowest CVD death rates.

Other population and transmigration studies also attest to the importance of dietary factors in affecting CVD mortality rates [10-12]. Comparison of Japanese living in Japan, Hawaii, and California clearly documented the importance of lifestyle in the pathogenesis of CVD as shown in table 2 [12].

Dietary Intervention Trial Evidence
Many studies of cholesterol lowering by dietary means have been carried out. In 1946, Morrison initiated a dietary trial consisting of an experimental group of 42 men and 8 women, who were survivors of a myocardial infarction (MI), and a control group of 43 men and 7 women [13, 14]. Alternate patients were placed on a low fat (25 g), low cholesterol (50-70 mg/day) diet and were followed for 3 years. Patients in the experimental group were found to have significant reductions in total cholesterol levels as well as less CVD mortality at the 12 year

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects number</th>
<th>Age (years)</th>
<th>Design</th>
<th>Randomized</th>
<th>Duration (years)</th>
<th>TC Change</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finnish Mental Hospital</td>
<td>5115 men, 5497 women</td>
<td>&gt;15</td>
<td>Crossover</td>
<td>No</td>
<td>6</td>
<td>-12-18</td>
<td>53% decrease in CVD mortality in men (p&lt;0.001); 34% decrease in women (ns)</td>
</tr>
<tr>
<td>Oslo Diet-Heart</td>
<td>412 men with CVD</td>
<td>30-64</td>
<td>Unblinded</td>
<td>Yes</td>
<td>5</td>
<td>-14</td>
<td>33% decrease in CVD mortality (p=ns)</td>
</tr>
<tr>
<td>Los Angeles VA</td>
<td>864 men with CVD</td>
<td>50-89</td>
<td>Double-blinded</td>
<td>Yes</td>
<td>5-8</td>
<td>-13</td>
<td>31% decrease in the endpoints of MI, CVD mortality, CVA, ruptured aneurysm, ischemic gangrene (p&lt;0.01); 20% reduction 1^st endpoints of MI and sudden death (p=NS)</td>
</tr>
<tr>
<td>Minnesota Mental Hospital</td>
<td>9057 men and women</td>
<td>all</td>
<td>Double-blinded</td>
<td>Yes</td>
<td>&lt;4.5</td>
<td>-14</td>
<td>No significant differences or trends were noted in MI or sudden death</td>
</tr>
<tr>
<td>Diet and Reinfarction Trial (DART)</td>
<td>2033 men with CVD</td>
<td>&lt;70</td>
<td>Factorial</td>
<td>Yes</td>
<td>2</td>
<td>-2.8‡</td>
<td>29% decreased in 2-year all-cause mortality in subjects advised to eat fish or who used 2 fish oil capsules/day; 33% reduction in CVD death (p&lt;0.01)</td>
</tr>
<tr>
<td>Lyon Diet Heart</td>
<td>605 men and women with CVD</td>
<td>&lt;70</td>
<td>Single-blinded</td>
<td>Yes</td>
<td>5</td>
<td>-7.5</td>
<td>65% decrease in CVD mortality in post-MI patients fed with alpha linolenic-rich diet (p&lt;0.01)</td>
</tr>
<tr>
<td>GISSI</td>
<td>11324 men with CVD</td>
<td>not reported</td>
<td>Factorial</td>
<td>Yes</td>
<td>3.5</td>
<td>+7-9</td>
<td>15% decrease in relative risk for all-cause mortality due to decrease in CVD death</td>
</tr>
</tbody>
</table>

In the above table, the % change in plasma total, rather than low density lipoprotein, cholesterol is reported due to the unavailability of the latter value in the early intervention trials. The † values are for the periods of 1959-1965 and 1965-1971, respectively. The ‡ represents decrease in those given fat advice only (p=NS). No changes from baseline were noted in either the fiber or fish advice groups. Abbreviations are: DART is the Diet and Reinfarction Trial; GISSI is the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardia; CVD, coronary heart disease; TC, total cholesterol; NS, not significant; MI, myocardial infarction; CVA, cerebrovascular accident.
follow up. A number of other relatively small trials were conducted between the years of 1956
1965, similarly concluding that restriction of dietary fat and cholesterol, under controlled
conditions, significantly reduced CVD mortality [15-21]. After 1965, the results of a number of
other dietary intervention trials using CVD morbidity and/or mortality as endpoints were reported
(summarized in table 3). These studies were larger than those reported in earlier years. The
Finnish Mental Hospital Study employed a cross over design and involved more than 10,000
male and female inpatients at two mental hospitals [22-24]. The experimental diet, in which milk
was replaced by an emulsion of soybean oil in skim milk and butter by margarine, resulted in 12
18% mean reductions in plasma total cholesterol levels. In men, CVD mortality was reduced
53% (p<0.002) by the experimental diet, whereas a 34% reduction was observed in women
(p=NS).

The first Oslo Diet Heart Study involved 412 survivors of an MI, aged 30 to 64, who were
randomized to a diet low in saturated fat (8.4% of energy) and cholesterol (264 mg/day) but high
in polyunsaturated fat (15.5% of energy), with 28% of total calories being derived from soybean
oil, rich in linoleic acid [25]. After 5 years of follow up, the intervention group had a mean
decrease in total cholesterol of 14%, a 33% reduction in MIs (P<0.05) and a 26% decrease in
CVD mortality.

The Los Angeles Veterans Administration Study also employed a relatively high
polyunsaturated fat diet [26]. In this trial, men living in a veterans’ home were randomized to
either treatment or control groups. The design of the treatment diet involved substitution of
vegetable oils (corn, cottonseed, safflower, soybean) for about two-thirds of animal fat. The two
groups were fed a diet containing 40% of total energy as fat with the treatment diet containing
time more polyunsaturated fat (16% of energy) and 40% less cholesterol (365 mg/day)
than the control diet. Saturated fat intake was approximately 11% of energy in the treatment
group and 18% of energy in the control group. A trend in favor of the treatment group for the
primary endpoints of MI and sudden death was observed. When other atherosclerotic events
were included, dietary treatment was found to be of significant benefit in CVD risk reduction (-
31%, p<0.01).

Another trial of significantly greater size, the Minnesota Coronary Survey, also assessed diets of
comparable total fat content [27]. This double-blind, randomized trial involved greater than
9,000 patients at six mental hospitals and one nursing home in Minnesota. Treatment and
control diets derived 39% of energy from fat, but the former diet was relatively enriched in
polyunsaturated fat (15% vs. 5%) and lower in saturated fat (9% vs. 18%) and cholesterol (166
vs. 446 mg/day). Despite a mean 14% reduction in plasma cholesterol levels, no significant
differences were noted in the study’s primary endpoints of MI and sudden death. The negative
results may have been due, in part, to the low mean cholesterol of subjects (207 mg/dL or 5.3
mmol/L) at baseline, as well as to the low mean age of study participants [27].

In the Diet and Reinforcement Trial (DART), 2033 men who had recovered from a myocardial
infarction were allocated to receive or not to receive advice on each of three dietary factors: 1) a
reduction in fat intake with an increase in the ratio of polyunsaturated to saturated fatty acids, 2)
an increase in fish intake, and 3) an increase in cereal fiber intake [28]. It should be noted that
members of the fish group could consume fish oil capsules (2 one-gram capsules per day) as a
partial, or total, substitute for fatty fish. A net reduction of 2.8% in total cholesterol was
observed in the group that received advice on fat intake over the 2-year period whereas no
differences were noted in either the total or HDL cholesterol levels of the fish and fiber advice
groups. Decreased mortality was not seen in those advised on either fat or fiber. In contrast,
total mortality was reduced by 29% (p<0.05) in those advised to increase fish intake relative to
those not advised with the difference being entirely attributable to a decrease in CVD deaths.
The Lyon Diet Heart Study, a randomized secondary prevention trial, compared the effects of a Mediterranean diet enriched in α-linolenic acid to a diet similar to that of the American Heart Association diet in MI survivors [29]. The experimental group, comprised of 302 men and women, consumed significantly less saturated fat, linoleic acid, and cholesterol, but more oleic and α-linolenic fatty acids than did the 303 men and women of the control group. A canola oil-based margarine (5% α-linolenic acid, 5% 18:1n-9 trans, 15% saturated fatty acids, 16% linoleic acid, and 48% oleic acid) was supplied to all members of the experimental group. Serum lipids, blood pressure, and body mass index remained similar in the experimental and control groups during the 2-year trial, with the trend with time being a decrease in LDL cholesterol and an increase in HDL cholesterol in the experimental group. After a mean follow-up of 27 months, there were 3 cardiac deaths in the experimental group relative to 16 in the control group. After adjustment for prognostic variables, this difference represented a 76% decrease in risk of cardiac death (p<0.02) although the number of cardiac deaths was small. The final results of the Lyon Diet Heart Study were subsequently reported by de Lorgeril and colleagues with a mean follow-up of 46 months [30]. These data confirmed those of the 2-year follow-up with the protective effect of the Mediterranean dietary pattern maintained up to 4 years after the initial infarction. Specifically, six cardiac deaths occurred in the experimental group relative to 19 in the control group, a finding translating into a 65% reduction in risk of cardiac death (p<0.01).

The independent and combined effects of n 3 PUFAs and vitamin E supplementation on morbidity and mortality in MI survivors were examined in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) Prevenzione trial [31]. Patients surviving a recent Mi (≤ 3 months) were randomly assigned to one of the following dietary supplement groups: 1) 1 g per day of n 3 PUFA (n=2836); 2) 300 mg per day of vitamin E (n=2830); 3) 1 g of n 3 PUFA + 300 mg of vitamin E per day (n=2830); or 4) control (n=2828). The dietary supplements, which were consumed by patients for approximately 3.5 years, were provided in capsule form. The primary combined efficacy endpoints were the cumulative rate of all cause death, nonfatal MI and nonfatal stroke. Compared with baseline values, there were no significant changes in plasma total, LDL, or HDL cholesterol levels in any of the groups; however, a small decrease (3.4%) in plasma triglycerides in the group receiving n 3 PUFA capsules without vitamin E was statistically significant. The analysis of study data revealed that treatment with n 3 PUFA capsules over a 3.5 year period was associated with a significant reduction (15%) in relative risk for the combined endpoints. Benefit was attributable to a decrease in the risk of all cause death, as well as cardiovascular death (17%), with the combined treatment of n 3 PUFA + vitamin E yielding similar results to those for the group supplemented with n 3 PUFA alone. Conversely, no beneficial effects were observed in the group supplemented solely with vitamin E in this study or other studies.

Overall, these data suggest benefit from restricting saturated fat and increasing polyunsaturated fat, especially of the n3 type. These trials are shown in table 3.

**Dietary Intervention Trials Using Coronary Angiography as an Endpoint**

Dietary intervention trials using angiography as an endpoint have also demonstrated that reduction of plasma cholesterol concentrations is of great benefit with respect to coronary atherosclerosis as shown in table 4. The Lifestyle Heart Trial, conducted by Ornish and colleagues, yielded particularly dramatic results, likely attributable to its use of a very low fat, very low saturated fat, low cholesterol diet, along with exercise and meditation [32-34]. Patients in the Lifestyle Heart Trial were asked to consume a low-fat vegetarian diet. The diet included fruits, vegetables, grains, legumes and soybean products without caloric restriction. All oils and animal products were excluded from the diet, with the exception of egg white and nonfat milk or yogurt. The diet contained approximately 7% of energy as fat, 15-20% as protein, 70-75% as
complex carbohydrates and 12 mg/day of cholesterol. The intervention group also participated in an exercise and meditation program. In the experimental group, LDL cholesterol was reduced by 37% (P<0.01) whereas HDL cholesterol did not change significantly. The lack of HDL lowering was likely due to the significant 22 pound weight loss, which occurred in the experimental group. Patients in the experimental group reported a 91% reduction in the frequency of angina after 1 year while those in the control group reported a 165% increase in frequency [33]. After 1 year, average percentage diameter stenosis regressed from 43.6 to 41.9% in the experimental group, yet progressed from 41.6 to 43.8% in the control group (p<0.02). After 5 years, the value continued to decrease in the experimental group (-3.1 absolute percentage points), with further progression noted in controls (+11.8 absolute percentage points) (p=0.001) [34]. This study clearly demonstrates that lifestyle changes can cause regression of coronary atherosclerosis.

Both the Cholesterol Lowering Atherosclerosis Study (CLAS) [35] and the St. Thomas' Atherosclerosis Regression Study (STARS) [36] have shown that cholesterol lowering through diet can inhibit the progression of atherosclerosis. CLAS was a randomized, placebo controlled, angiographic trial that examined 162 men, aged 40 50, with progressive atherosclerosis who had undergone coronary bypass surgery [35]. Dietary goals were to provide 26% of total energy as fat, with <5% as saturated fat, <10% each as monounsaturated and polyunsaturated fats, and <250 mg/day of cholesterol within the context of a self-selected diet. Data analysis of 24 hour dietary recalls, as well as assessment of angiograms, revealed that increased consumption of either total fat or saturated fat was associated with a significant increase in risk of new lesions. Subjects in the diet phase of CLAS in whom new lesions did not develop had increased dietary protein to compensate for reduced fat intake by substituting low fat meats and dairy products for high fat meats and dairy products. These results suggest that protein and carbohydrate are reasonable replacements for saturated fat in the diet.

STARS was designed to assess the effects of a practical lipid lowering diet on the coronary arteries of patients with CVD [36]. In the lipid lowering diet, total fat intake was reduced to 27% of dietary energy, saturated fat to 8-0% of energy, polyunsaturated fat to 8% of energy, and cholesterol reduced to 100 mg/1000 kilocalories. Subjects in the intervention group had significant reductions (p<0.01) in total cholesterol (14%), LDL cholesterol (16%), and triglyceride (20%) concentrations, with no significant differences in HDL cholesterol. Approximately 3 years after randomization, computerized image analysis of coronary arteries revealed that dietary change had not only retarded progression of CVD in 15% of subjects in the intervention group but had caused overall regression in 38% as well. Mean percentage diameter stenosis decreased 0.5% in the intervention group whereas it increased 5.6% in the control group (p<0.001).

Therefore, dietary intervention trials have evaluated the effects of reduced intake of total and saturated fat, or increased intake of PUFAs, both n-6 and n-3 PUFAs, on cardiovascular
morbidity and mortality. The results of these trials, as well as those assessing angiographic end points, clearly demonstrate that restriction of dietary saturated fat along with increases in the consumption of the essential n-6 and n-3 fatty acids (especially n3 fatty acids) reduces CVD risk and decreases the rate of progression of coronary atherosclerosis and in some cases promotes regression. Conversely, the results of randomized prospective placebo controlled trials examining the cardio protective effects of antioxidants, predominantly vitamin E, do not support the concept that vitamin E, beta carotene, or the combination of these two antioxidants along with vitamin C reduces CVD risk.

Data in the above tables are consistent with the concept that aggressive lifestyle modification especially if accompanied by weight loss as seen in the Lifestyle Heart Study can have a significant impact on remodeling of atherosclerotic plaque in coronary arteries as assessed by angiography.

**Dietary Recommendations for Coronary Heart Disease Prevention**

The recommendations of the third Adult Treatment Panel of the National Cholesterol Education Program are shown in table 5 [5]. The cornerstone of these recommendations is restriction of saturated fat to less than 7% of calories and cholesterol to less than 200 mg/day.

There remains considerable confusion about which is the optimal vegetable oil to consume. The ideal vegetable oil is one that is very low in saturated fat and high in essential fats especially omega 3 fatty acids such as alpha-linolenic acid. Canola oil has all these characteristics.

The current dietary recommendations of the World Health Organization for the prevention of chronic disease are shown in table 6.
The USDA recommendations have recently been revised to include decreased intake of bread, cereal, rice or pasta and increased intake of vegetables, fruits and essential fats [37]. Please see www.mypyramid.gov. The recommendations were for 6-11 servings/day of bread, cereal, rice, or pasta, 3-5 servings of vegetables/day, 2-4 servings of fruits/day, 2-3 servings of milk, yogurt, or cheese per day, 2-3 servings of meat, poultry, fish, dried beans, eggs, or nuts, and to use fats, oils, and sugars sparingly.

1.3.3.2 Clinical significance of weight-related medical problems

Weight-related medical problems are among the most serious health problems facing the United States (US). Approximately 60% of adults and 30% of school-age children in the US are overweight [body mass index (BMI) > 25 kg/m²], and approximately half of overweight adults and children are obese (BMI ≥ 30 kg/m²)[38, 39]. CVD, the leading killer of men and women in the US and abroad, is twice as likely to occur in obese as in normal weight individuals, and all metabolic cardiac risk factors, including hypertension, T2D, and dyslipidemias, are substantially exacerbated by obesity [40, 41]. Increasing levels of BMI predict progressively shortened lifespan [42], and approximately 300,000 US deaths per year are attributable to obesity [43]. Excess body weight is also associated with worsened self-esteem, vitality, bodily pain and other quality of life indicators [44]. The US Surgeon General has identified obesity as a top health threat for the US [45] and reversal of excess body weight is one of the top health priorities of the US government [46].

1.3.3.3 Economic significance of weight-related medical problems

Obesity has overtaken smoking as the most costly source of health problems [47]. Weight-related medical problems have been estimated to cost the US over $75 billion annually in direct health care expenditures [48, 49].

Table 5: National Cholesterol Education Program Guidelines on Therapeutic Lifestyle Modification

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Average U.S. Diet</th>
<th>Therapeutic Lifestyle Modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Fat</td>
<td>34%</td>
<td>25-35%</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>12%</td>
<td>&lt; 7%</td>
</tr>
<tr>
<td>Monounsaturated Fat</td>
<td>13%</td>
<td>&lt; 20%</td>
</tr>
<tr>
<td>Polyunsaturated Fat</td>
<td>7%</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>Cholesterol (mg/day)</td>
<td>270</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>Total Energy Calories</td>
<td>-</td>
<td>To achieve and maintain desirable body weight</td>
</tr>
</tbody>
</table>

* % of total energy. †Total population data from National Health and Nutrition Examination Survey (NHANES) III, excluding children under 2 years of age

Table 6: World Health Organization Population Nutrient Intake Goals

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat</td>
<td>15-30% of total energy</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>&lt;10% of total energy</td>
</tr>
<tr>
<td>N6 polyunsaturated fats</td>
<td>5-8% of total energy</td>
</tr>
<tr>
<td>Protein</td>
<td>10-15% of total energy</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td></td>
</tr>
<tr>
<td>Complex</td>
<td>55-75% of total energy</td>
</tr>
<tr>
<td>Simple</td>
<td>&lt;10% of total energy</td>
</tr>
<tr>
<td>Fiber (nonstarch polysaccharides)</td>
<td>16-24 g/day</td>
</tr>
<tr>
<td>Fruits and vegetables</td>
<td>&gt;400 g/day</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>&lt;5 g/day</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>&lt;300 mg/day</td>
</tr>
</tbody>
</table>

Lifetime excess medical costs for individual patients with obesity have been estimated to be over $10,000 [50]. Some studies have shown that obese employees have 60% higher risk of excess absenteeism than their normal weight counterparts [51] and cost their employers over $750 per year in excess medical expenditures [52]. The high cost of obesity stems mainly from increased prevalence of expensive chronic medical problems. For example, obesity accounts for approximately 35% of heart disease, 45% of hypertension and 85% of T2D cases and their associated health costs [53].

1.3.3.4 Acceleration of weight-related medical problems

Obesity is spreading throughout the world at an alarming rate. In the past 3 decades, the prevalence of obesity in the US has more than doubled in adults [54] and nearly tripled in children [55]. This “epidemic” of obesity has affected every US state [56], racial group and both genders with similar trends throughout the world [56, 57]. The prevalence of T2D has been rising throughout the US [58], with rates projected to increase from 4.4% now, to 9.7% in 2050 according to one Markov analysis [59]. Previously rare in adolescents, T2D now comprises 8-45% of newly diagnosed cases of diabetes in US children [60]. Obesity and diabetes have exacerbated a global epidemic of cardiovascular disease [61] and are jeopardizing five decades of a decrease in CVD rates in the US [62].

1.3.3.5 Dietary changes reduce weight-related medical problems

Sustained dietary changes can favorably impact diet-related medical problems especially in overweight patients. Among adherent individuals, sustained weight losses of over 10% of initial body weight are commonplace while participating in diet and lifestyle modification programs. Mean weight losses of around 3-5% at 1 year are typical of intent-to-treat protocols. The modest weight losses observed in such programs typically improve and can sometimes normalize hyperglycemia, dyslipidemia and hypertension [63-68]. A 5% weight loss typically reduces a given cardiac risk factor by 10%, a finding demonstrating that modest weight losses have a magnified effect on risk factors. Furthermore, modest weight losses substantially improve quality of life according to several measures. Modest weight loss of approximately 3% of body weight resulting from behavioral programs has been estimated to be a cost-effective, efficient use of resources ($25-50K per quality-adjusted life-year) according to an AHRQ-sponsored analysis submitted to the US Congress for consideration of subsidizing weight loss programs for Medicare patients.

Longer-term changes in diet and exercise are achievable and can prevent T2D [69] and CVD events [70, 71]. In the Diabetes Prevention Program, T2D incidence was 58% lower in a lifestyle improvement group that sustained a 4-7% weight loss for a mean of 2.8 years compared to a control group with no weight loss [69].

1.3.3.6 Health benefits of starch-based foods

The health benefits of starch-based foods appear to depend on several factors, particularly the fiber content, glycemic index and caloric density [72-74]. The notion that the health benefits derived from unrefined grains are superior to refined grains is practically undisputed in nutrition circles [75]. Intervention studies demonstrate that high fiber versus low fiber grain products are beneficial for weight loss and lipid profiles [76]. The importance of glycemic index, a measure of the post-prandial effects of a standard carbohydrate quantity on blood glucose levels has been debated extensively [77-79]. Observational studies indicate that glycemic load, the glycemic index multiplied by the total carbohydrate quantity, has been associated with heart disease, diabetes risk and several cancers in a dose-dependent fashion [80-88]. Intervention studies comparing low versus high glycemic loads suggest that glycemic load reduction favorably affects hunger, body weight, lipid profiles and diabetic control [89-91]. Caloric density, an
important determinant of caloric intake, ranges widely for starch-based foods [81]. Unfortunately, most starch-based foods consumed in the US are highly refined, with low fiber and high glycemic index [92, 93]. Furthermore, starch-based foods have been major vehicles for unhealthy fats [94, 95]. Saturated and hydrogenated fats are commonly (if not primarily) found in processed starch-based foods including muffins, crackers and cookies. Despite the likely benefits of cereal fiber, promotion of a high starch diet may have inadvertently promoted a high calorie, appetite-stimulating diet low in fiber and excessively high in glycemic load and hydrogenated fat.

1.3.3.7 Health benefits of fruits and vegetables
The health benefits of vegetables and fruits have been demonstrated. Epidemiological studies strongly support the notion that vegetables and fruits are favorable for prevention of cancer and heart disease [96, 97] with support from randomized trials as well [98-102]. For example, the Dietary Approaches to Stop Hypertension (DASH) trial demonstrated the benefits of a diet high in fruits and vegetables (which was further augmented by increasing healthy protein such as fish, low-fat dairy, and nuts) for reducing blood pressure and other CVD risk factors [103-105]. In general, vegetables and fruits have favorable ratings for caloric density, glycemic load, fiber content, micronutrient levels and antioxidants. The effects of replacing starch-based foods with vegetables and fruits are largely unknown. In general, many experts and agencies are eager to increase consumption of vegetables and fruits throughout the US and abroad [106-113].

1.3.3.8 Health benefits of various protein and fat sources
The health benefits of protein-based foods vary according to source and fat content. Fish is one of the few foods proven to delay mortality in patients with CVD in part due to reductions in fatal arrhythmia and strokes. Omega-3 fatty acids are purported to be a major source of the benefits. Mercury and other toxins can accumulate in fish, but the benefits are likely to outweigh the harms if fish is consumed in moderation. High saturated fat varieties of red meat, poultry and dairy foods are clearly unfavorable although very low-fat versions of these protein sources have appeared favorable in several epidemiological and intervention studies of heart disease risk factors [114, 115]. Soy protein has a mildly favorable effect on lipid profiles compared to protein from red meat and poultry [116-119], and several health agencies now promote the consumption of soy protein [120]. The clinical effects of estrogen-like compounds in soy foods are being studied including the risk of stimulating estrogen-sensitive tumor cells [121-123]. Other beans and legumes have lower protein concentrations but are also considered to be important protein sources with favorable health effects [124]. Nuts have fared well in epidemiological and intervention trials [125-128] and may have favorable enough effects on hunger to offset their high caloric density values [129, 130]. Monounsaturated fats, which comprise most of the calories in nuts, are one of the purported sources of such health benefits [130]. The relative benefits of polyunsaturated fats found in many cooking oils have been hotly debated, and benefits depend on which foods they replace and what health parameters are studied [131].

1.3.3.9 Effects of replacing dietary carbohydrate with protein
Moderate replacement of typical dietary carbohydrates with lean protein improves all lipid parameters (especially triglyceride levels) [132-134], reduces insulin and glucose [135], and enhances weight loss associated with reduced fat diets [136, 137]. Data from the Nurse’s Health Study suggest that reduced risk of cardiovascular disease is associated with replacement of carbohydrates with lean protein. In the Cholesterol Lowering Atherosclerosis Study, progression of coronary lesions was prevented more effectively by replacement of dietary fat with lean protein rather than carbohydrates. Increased protein diets have been criticized because of concerns about increased risk of renal compromise or osteoporosis. In
contrast, except in subjects with pre-existing renal disease, moderate protein increases do not appear to adversely affect renal function. Replacement of carbohydrate with protein may actually retard bone loss, and increased protein intake is associated with reduced incidence of hip fracture in elderly women [138-142].

1.3.3.10 Prospective studies to improve the USDA food pyramid

The USDA food pyramid is the most highly recognized source of “official” dietary advice. The USDA food pyramid has been based on the best evidence available from nutrition research studies, but competing versions of food pyramids have not been tested prospectively to determine their health effects on the target population. In contrast, many authorities have commented on the controversy surrounding the USDA food pyramid or suggested modifications [143-155]. Prospective testing of food pyramids must be done given the legitimate controversies surrounding its design and failure to control the obesity and diabetes epidemics. As a top research priority item, the 2005 Dietary Guidelines Advisory Committee Report specifically calls on investigators to “conduct clinical trials to determine the effect of intake of foods from various commodity food groups or whole diets on BMI, lipid metabolism, cardiovascular disease, T2D, cancer and osteoporosis” [156, 157].

1.3.3.11 Preliminary Studies by the Study Investigators:

Investigators: Dr. Francine Welty, the principal investigator, has an M.D. and Ph.D. in biochemistry. Her clinical training has been in internal medicine and cardiology, and she has been a staff cardiologist at the Beth Israel Deaconess Medical Center since 1991. She has had a strong research interest in the area of lipoprotein metabolism as well as in the area of HDL deficiency. Dr. Welty’s first major contribution was the discovery of a new mutation leading to hypobetalipoproteinemia, namely the apoB-67 kindred [158]. This is a truncated form of apoB, which leads to low levels of LDL cholesterol, and apparently protection from heart disease. She has carefully defined the genetic mutation [159] and using stable isotopes, she has shown that LDL-C levels are low due to decreased production of apoB [160]. Moreover, Dr. Welty has carefully documented the genetic causes of low LDL in the general population, and has carried out a significant number of stable isotope kinetic studies with Dr. Schaefer’s group examining the regulation of apolipoprotein metabolism [160-180].

Most importantly for this study, Dr. Welty has carefully developed a nutritional intervention program known as The Heart and Soul Program, which consists of 12 classes for patients with heart disease to modify their diets, exercise and promote weight loss [181]. In the study Dr. Welty documented that in the free living state with an intensive lifestyle program including dietary modification and exercise, one could substantially reduce body weight and lower LDL cholesterol without lowering HDL cholesterol. In addition, significant improvements in exercise tolerance were achieved [181]. A total of 27 CVD patients completed this 12 week dietary and exercise instruction program and were followed for 6 months. Based on food record analysis, caloric intake decreased 19%, total fat 42%, and saturated fat by 54%, while body weight went down by 4.4%, and weekly exercise increased from 98 to 216 minutes/week. These changes were accompanied by a 57% decrease in anginal episodes [181]. Moreover, LDL cholesterol decreased 10%, HDL cholesterol increased 3%, triglycerides decreased 19%, the total cholesterol/HDL cholesterol ratio decreased 11%, and systolic and diastolic blood pressure decreased by 9% and 13%, respectively [181]. This program will serve as an important model for the current project. In addition, Dr. Welty has been studying other dietary constituents, including whole soy nuts and has documented reduction in blood pressure as well as LDL cholesterol in hypertensive, hyperlipidemic postmenopausal women. She also has carefully reviewed the literature on the role of nutrition and hormonal replacement in CVD prevention in women [181-190].
In addition, Dr. Welty has collaborated with Dr. Schaefer and has done very careful metabolic studies showing that there is an important interrelationship between apoA-I catabolism and apoB-48 [180](231). Dr. Welty also has a long record of accomplishment as an established clinical investigator and has participated in a variety of clinical studies. She served on the endpoint committee of the recently-completed Treating to New Targets study in which 10,000 patients worldwide with heart disease were randomized to atorvastatin 10 mg versus atorvastatin 80 mg per day. She has also been a member of the Steering Committee for the BELLES study, which involved an examination of cardiac calcification in postmenopausal women treated with Lipitor versus Pravachol. She is also a member of the Steering Committee and Endpoint Committee for PEARL, a study of a selective estrogen receptor modulator in women. She is also a member of the Endpoint Committee for ILLUMINATE, the new clinical endpoint committee for Lipitor alone versus Lipitor plus Torcetrapib, and the endpoint committee of SPARCL, a stroke study. Therefore, she has a long and successful record as a clinical investigator. Dr. Welty is also the principal investigator for the NIH-funded Nutrition Academic Award at Harvard Medical School.

On this dietary project, Dr. Welty and Dr. Schaefer will be Co-Principal investigators and work closely with colleagues at the Lipid Metabolism Laboratory of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in Boston [70]. Dr. Schaefer will be directing the central laboratory efforts with the assistance of Dr. Bela Asztalos (see core laboratory description).

Dr. Schaefer’s laboratory served as the central laboratory for the Veterans Affairs High Density Lipoprotein Intervention Trial (VA-HIT) in which 2500 men were randomized to gemfibrozil versus placebo [191-193]. These men were selected for having heart disease and low HDL cholesterol. In these studies, it was documented that gemfibrozil exerted its beneficial effect mainly in those with elevated insulin levels as well as by lowering CRP and raising HDL cholesterol. One of the most important features to emerge from this study is that, if one selects men for heart disease and low HDL cholesterol (i.e. < 40 mg/dl), one tends to get men who are obese and have elevated insulin levels, strongly underlying the link between visceral adiposity, liver lipid, insulin resistance and HDL deficiency. Dr. Schaefer’s laboratory is currently serving as the Central Laboratory for another VA study in 1800 subjects with diabetes comparing tight with less tight control of HBAIC and fasting serum glucose levels.

Dr. Schaefer’s group also has expertise in carrying out outpatient dietary studies. He and Dr. Michael Dansinger completed a 1 year evaluation of popular diets in 160 obese subjects documenting that all four popular diets (Atkins, Ornish, Weight Watchers, and Zone) could promote weight loss and CVD risk reduction, and that self-rated compliance with individual programs (book plus 4 classes) was the best predictor of weight loss [194]. Moreover he has just completed a 5 year NIH funded effort in which he has documented the benefits of low glycemic load(GL) diets in promoting more significant long term (one year and 22 weeks) weight loss (-4.6%) and reduction in non-fasting insulin levels (-40%) in 80 obese individuals than high GL diets (-2.5% and +3%) (unpublished observations).
1.3.4 Background specific for Salsalate

1.3.4.1 Overview of NF-κB signaling:

Nuclear Factor-Kappa B (NF-κB) is a central integration site for pro-inflammatory signals and a master regulator of genes involved in inflammation, innate immunity, and apoptosis. NF-κB is composed of homo- and heterodimers of five members of the Rel family, including NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, and c-Rel (Rel). Numerous inputs that activate NF-κB in addition to pro-inflammatory cytokines include bacterial cell wall and viral products, dsDNA, mitogens, and oxidative stress [195, 196] (Figure 3). NF-κB dimers are sequestered in the cytosol of unstimulated cells via non-covalent interactions with a class of inhibitor proteins, called I-KappaB’s (IκB) (which are also complex proteins composed of IκBα, IκBβ, IκBγ, IκBε, BCL3, p100, and p105). The degradation of IκB proteins permits NF-κB molecules to move into the nucleus where NF-κB mediates transcription of large numbers of target genes. IκB degradation is carried out by the proteasome but only after phosphorylation by the IκB Kinase Complex (IKK). The IKK complex is composed of three subunits: two, IKKα (IKK1) and IKKβ (IKK2), which are bona fide kinases, while the third, IKKγ (NEMO), has no catalytic activity but plays a critical regulatory role. IKKα is the predominant I-KappaB kinase.

Activation of the IκB kinase (IKK) complex promotes IκBα phosphorylation at S32 and S36, ubiquitination, and subsequent proteosomal degradation. This releases NF-κB from IκB permitting translocation of NF-κB into the nucleus and where it mediates transcription of large numbers of target genes (see Gilmore’s website for a reasonably comprehensive list, http://people.bu.edu/gilmore/nf-κb/target/index.html). The partial list of target genes in Figure 4 includes many that have been implicated as markers or mediators of atherosclerosis, insulin...
resistance and T2D. IKKβ is the component of the IKK complex primarily responsible for activating the classical NF-κB (p50/RelA) pathway [195-197].

NF-κB drives the production of multiple gene products that are potentially relevant to the atherosclerotic process, including PAI-1, CRP, inducible nitric oxide synthase (iNOS), as well as COX2, IL-1β, IL-6 and TNF-α ligands and receptor. Furthermore, IKKβ can directly inhibit insulin signaling by serine phosphorylation of important insulin signaling proteins, such as the insulin receptor substrate-1 (IRS-1) [198].

Recently, high fat diets and obesity have been shown to lead to a low-grade, sub-acute activation of NF-κB in adipose and liver tissues in rodents [199] providing an important link between acquired obesity and inflammation.

**Inhibition of IKKβ/NF-κB using salicylates:**

High levels of anti-inflammatory salicylates, including aspirin and salicylic acid, have been demonstrated to inhibit NF-κB [200] by inhibiting IKKβ [201]. We propose that IKKβ and NF-κB might be involved in the pathogenesis of the metabolic syndrome including insulin resistance, atherosclerosis and T2D. We provide preliminary data in animal models of diabetes and in insulin resistant persons that salicylates improve insulin resistance, reduce free fatty acids and the inflammatory protein CRP [199, 202, 203]. We propose to evaluate the effects of IKKβ/NF-κB inhibition on vascular remodeling in persons with coronary artery disease and thereby provide new potential targets for treatment of these conditions.

Our collaborator Dr. Shoelson has conducted numerous in vitro (biochemical, cell biology) and in vivo (transgenics, knock-outs, genomic, pharmacologic) studies that identify the IKKβ/NF-κB pathway as the molecular target for salicylates as agents for treating T2D and insulin resistance [199, 202-206].

Salicylates have been shown to improve insulin sensitivity and glucose tolerance in obese rodents [199]. These metabolic changes are associated with a marked reduction in circulating triglyceride and free fatty acid levels. High fat diets and lipid infusion induce insulin resistance, and both salicylate treated and IKKβ gene knockout animals are protected from these deleterious effects [202]. Furthermore, IKKβ knockout mice are protected against diet-induced obesity, demonstrating the importance of this pathway in the inflammation occurring in the setting of the epidemic of obesity.

Salicylates are widely prescribed anti-inflammatory agents. Their effectiveness has largely been attributed to their ability to inhibit prostaglandin production by inhibiting the cyclooxygenase, prostaglandin H (PGH) synthase. Low doses of aspirin, 81 mg daily, inhibit cyclooxygenase-1 (COX1) thereby inhibiting platelet aggregation and are used to prevent thrombosis associated with atherosclerosis. Two aspirin (650 mg) can inhibit both COX1 and COX2, and are commonly used for relief of pain such as headache. Many other non-steroidal anti-inflammatory drugs (NSAIDS) similarly inhibit the cyclooxygenases. However, doses of aspirin necessary to treat chronic inflammatory diseases are much higher than those required to inhibit prostaglandin synthesis. More recently NF-κB was identified as the molecular target of high dose salicylates through inhibition of the activity of IKKβ [200, 201]. This effect of salicylates to inhibit NF-κB is not seen with other nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin [200]. Likewise, TNFα inhibition of insulin signaling is reversed by salicylates, but not by COX inhibitors including Ibuprofen, Naproxen or specific COX2 inhibitors [199].

To demonstrate that the use of salicylates in the treatment of diabetes is not new, the utility of sodium salicylate in diabetes was first reported in 1876 [1, 207], and beneficial effects of high-
doses of salicylate or aspirin have been recurrently rediscovered since then [3, 208-210]. In a reinvestigation of its anti-diabetic effects, high-dose aspirin (acetylsalicylic acid) (~ 7 g/day) was recently shown to substantially improve circulating glucose, triglyceride and free fatty acid levels as well as hepatic glucose production and glucose utilization in patients with T2D [203]. However, the therapeutic potential of high-dose aspirin is limited by adverse side effects, including bleeding, gastric irritation and ulcer, and tinnitus. In addition to inhibition of IKKβ/NF-κB pathways, aspirin inhibits the cyclooxygenase enzymes COX1 and COX2 through covalent transacetylation of their active sites. The COX inhibition leads to diminished platelet aggregation and with the gastric irritation can lead to life threatening bleeding complications.

Importantly, aspirin (acetylsalicylic acid) contains acetyl group that is necessary for COX inhibition. In contrast, sodium salicylate does not contain an acetyl group and thus, is a much weaker inhibitor of COX1 and COX2 than aspirin, and therefore is not associated with a risk of bleeding, but is of equivalent potency towards IKKβ and NF-κB [200, 201].

We therefore proposed that salicylic acid might be an effective and safer alternative to aspirin in the treatment of insulin resistance and the metabolic syndrome. Salsalate (DisalcidTM) is a pro-drug form of salicylic acid (2-hydroxybenzoic acid 2-carboxyphenyl ester) that is not associated with gastrointestinal bleeding or a prolonged bleeding times, even at comparably high salicylate doses [211, 212]. Therefore, we have evaluated the effects of the salsalate on insulin sensitivity and insulin secretion in subjects with insulin resistance of T2D or impaired glucose tolerance (IGT) and report the results of this clinical trial in our preliminary data section.

1.3.4.2 Inflammation in the Metabolic Syndrome:

A growing body of evidence over recent years supports a potential role for cytokine-associated, subacute inflammation in the pathogenesis of insulin resistance and T2D [213, 214]. Inasmuch as insulin resistance is an underlying feature of the metabolic syndrome, subacute inflammation may also play a role in the development of associated dyslipidemias, hypertension and atherosclerotic process. Together, insulin resistance, with or without hyperglycemia, dyslipidemia, and hypertension all increase risk for atherosclerotic forms of cardiovascular disease, which is itself increasingly thought to be a disease of chronic subacute inflammation [215, 216]. These interrelationships suggest that inflammation may be the basis of a “common soil” [217] involved in the pathogenesis of both T2D and atherosclerosis.

Pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α, are produced in cells involved in immunity and inflammation, such as macrophages and monocytes, and in adipose tissue and liver, particularly in response to over-nutrition. The cytokines (IL-1β, IL-6 and TNF-α) also act on liver to produce a characteristic dyslipidemia associated with atherosclerosis and diabetes, increased VLDL and decreased HDL, and to promote the release of acute-phase proteins which are atherosclerotic risk factors, such as fibrinogen. Circulating cytokines may act directly on the endothelium to promote atherogenesis and may impair β-cell insulin secretion.

Elevations in components of the acute-phase response and of inflammation more generally have been shown quite convincingly to occur in T2D and predict risk for its occurrence [213, 214, 218-230]. Elevated markers include PAI1, CRP, fibrinogen, leukocyte count, sialic acid, IL-6 and IL-1β. All markers are also intimately involved in the atherosclerotic process. However, the big question in the field has been whether these are simply correlative markers for the process or causatively involved in the pathogenesis of the metabolic syndrome. It is clear that pro-inflammatory cytokines such as IL-6 and TNF-α can cause insulin resistance in model systems [231-233], but it has been difficult to make the leap to what actually occurs in patients with the disease.
Our findings with anti-inflammatory salicylates, presumably working as inhibitors of IKKβ and NF-κB, have provided new impetus to the field [199, 204]. We hypothesized that NF-κB activation, as a central mediator of inflammatory responses, might be a root of insulin resistance. The partial list of target genes in Figure 2 includes many that have been implicated as markers or mediators of atherosclerosis. Those that are highlighted by arrows have actually been reported to promote insulin resistance in animal models or human conditions [220, 222, 226, 227, 230, 231, 233-250]. Since any or all of these might promote resistance, decreased expression through NF-κB inhibition should sensitize in each case. We hypothesize that NF-κB orchestrates the transcription of a constellation of genes, some known and others yet to be discovered, that coordinately mediate obesity-induced insulin resistance and predispose to atherosclerosis. The corollary is that NF-κB inhibition coordinately down-regulates an entire constellation of putative mediators [199, 204, 218].

1.3.4.3 Inflammation and Cardiovascular Disease:

Inflammation is now recognized as an important component of the atherosclerotic process. In response to injury, circulating leukocytes and monocytes are attracted to the vessel wall by the release of chemotaxins, bind, and become macrophages and foam cells thereby participating in the development of the fatty streak and subsequently the atheromatous lesion. Activation of macrophages, T lymphocytes and smooth muscle cells lead to the release of additional mediators including adhesion molecules, cytokines and growth factors which all contribute to progression of atherogenesis. Interleukin-6 (IL-6) is a principle pro-coagulant cytokine, which can increase plasma concentrations of fibrinogen, plasminogen activator inhibitor type 1 (PAI1) and CRP [251]. In turn, the inflammatory cytokines can induce expression of cellular adhesion molecules, mediating adhesion of the leukocytes to the vascular endothelium and amplifying the process (reviewed in [252]). We propose that a subacute inflammatory process may be induced in fat and liver as a consequence of obesity and western diet and that the production of these activators leads to vascular damage, as well as insulin resistance.

Raised levels of cytokines and adhesion molecules including IL-6 [253], serum amyloid A [254]), TNF-α [255], ICAM [256], and CD40 ligand [257], among others, predict coronary events in healthy men and women, in patients with stable angina, acute coronary syndromes and in secondary prevention studies. Of the markers of systemic inflammation, CRP is most widely available to measure. CRP is an acute phase reactant that may increase up to 1000 fold in the setting of acute major infection. Yet more modest elevations predict future MI and stroke [258, 259]. Recent large scale studies in humans suggest that elevated levels of CRP are associated with higher frequency of cardiovascular events, and that lowering of CRP with statins can reduce the risk of cardiovascular events and reduce the rate of progression of atheroma, assessed by intravascular ultrasonography, independent of effects on lipids [260].

Salicylates can reduce CRP in patients with the metabolic syndrome and diabetes. High dose aspirin, about 7 grams daily, reduced CRP by 17% [203] and in our preliminary data we demonstrate that salsalate reduced CRP by almost 50%.

Furthermore, IKKβ mediates the impairment of nitric oxide production in endothelial cells associated with elevated free fatty acids [261]. Salicylates selectively inhibit IKKβ [201], and in our preliminary studies were associated with dramatic 30% reduction in circulating free fatty levels. Thus, the effect of salsalate to improve endothelial nitric oxide may be direct at the level of IKKβ or indirect via reductions in free fatty acids, but should improve vascular status.
1.3.4.4 Insulin Resistance and Cardiovascular Disease:

Insulin resistance is highly associated with T2D, as well as hypertension, dyslipidemia, obesity and cardiovascular disease itself [262]. Insulin resistance has been demonstrated to precede and predict both incident diabetes [263, 264] and cardiovascular disease [265, 266]. While recent studies demonstrate that improvement in glycemic burden reduce microvascular complications of diabetes, the effects on cardiovascular events has been disappointing. Thus, it is not surprising that many studies have evaluated the potential effects of insulin sensitizing agents on cardiovascular outcomes. In the United Kingdom Prospective Diabetes Study (UKPDS) the decrease in both cardiovascular risk and total mortality reach significance in a subgroup of overweight patients receiving metformin [267, 268] and carefully designed prospective studies are ongoing to evaluate the potential benefit of thiazolidinedione to prevent macrovascular disease [269]. Although both agents have been shown to have beneficial effects on blood pressure, lipid profiles [270, 271], microalbumin [272] and other markers of vascular risk including PAI-1 [273], CRP [274] and carotid arterial wall thickness [275-278], data on event rate reduction is not yet available. However, insulin sensitizing agents appear to have non-hypoglycemic effects to improve multiple independent predictors of coronary heart disease. Whether salicylates, via their anti-inflammatory and insulin sensitizing properties have similar effects is the main topic of this project, but preliminary data suggest that they lower glycemia and inflammatory markers that are often increased in obesity, diabetes and cardiovascular disease thus may have benefit for persons with atherosclerosis.

1.3.4.5 Treating Inflammation of the Metabolic Syndrome/Diabetes with Salicylates

Given a potential relationship between inflammation and diabetes, we asked whether anti-inflammatory agents might influence insulin resistance and glycemic control. Clinicians are well aware of the hypoglycemia often associated with aspirin overdose, but the mechanism is unknown and attributed in modern textbooks to liver damage or uncoupled oxidative phosphorylation [279, 280]. We rediscovered that salicylates had actually been used to treat diabetes, traceable to as early as 1875 [1] (Figure 5).

A report in 1957 showed a beneficial ‘side’ effect of high-dose aspirin used to treat a diabetic person with acute rheumatic fever [3], which prompted the authors to study 7 additional diabetic patients prospectively (Figure 6). Every patient responded to high-dose aspirin. The average fasting blood glucose value fell from ~200 mg/dl to less than 100 mg/dl during 14 days of treatment 5-8 g/day ASA. Fasting blood glucose levels crept back up after treatment was discontinued. Another study in 1960 similarly reported marked improvements in glycemic control [208]. Six hospitalized diabetics were taken off insulin and placed on a 1800-2000 kcal/d diet (Figure 7). Data were collected after 7 d diet alone, following ASA treatment (6 g/day x 10 day), and after a 5 day washout. Again, blood glucose values improved in every patient. Mean fasting blood sugar...
fell from before treatment values of 371 ± 116 mg/dl, to 128 ± 28 mg/dl during treatment, and increased again after patients were taken off aspirin treatment.

Thus, several historical studies clearly demonstrated that high-dose (5-10 g/day) aspirin and salicylate dramatically improved glycemic control. These old results from human studies closely match our recent ones in obese insulin resistant rodents - and show that salicylates can be as effective at lowering blood glucose as troglitazone or metformin. Side effects associated with high-dose aspirin (e.g. tinnitus, deafness, gastrointestinal distress), the narrow therapeutic window, and positive outcomes obtained with sulfonyleureas during the 1950's may have eclipsed interest in aspirin as an oral hyperglycemic agent. Nevertheless, it is not clear why salicylates have been neglected as an experimental tool throughout what we consider to be the entire modern era of mechanism-based drug discovery - it simply seems to have been forgotten.

The pharmacological findings in patients and target validation studies with rodents are briefly outlined in Preliminary Results. Most importantly for this application, we have reassessed the effects of high dose aspirin and salicylate in insulin resistant type 2 diabetic patients and seen impressive reductions in glucose, cholesterol, triglyceride and free fatty acid levels, and CRP. The purpose of the proposal is to expand these preliminary studies to look at the longer-term clinical efficacy of salicylate therapy in the related atherosclerotic complication of the metabolic syndrome. These studies will determine whether salicylates are viable treatments and in addition will validate the IKKβ/NF-κB pathway as a target for drug discovery efforts that aim to treat atherosclerosis.

1.3.5 Preliminary Results Using Salicylates in Diabetes

1.3.5.1 Aspirin in T2D

Since the preliminary trial to use high-dose ASA to treat nine patients with T2D has been published [203], a detailed summary of the findings is not provided. In brief, significant effects were seen on many metabolic parameters, including reductions in fasting (25%) and postprandial glucose (AUC, 20%), CRP (17%) total cholesterol (15%), triglycerides (50%), fasting (50%) and postprandial (AUC, 50%) free fatty acids and insulin clearance (30%). Clamp studies revealed reductions in hepatic glucose production (20%) and an improvement in insulin-stimulated peripheral glucose disposal (25%) [211].

1.3.5.2 Salsalate in T2D

Overview: In view of the dramatic improvements in insulin sensitivity, glycemia and other metabolic measurements documented with aspirin and the increased safety profile of salsalate, we initiated human clinical studies to evaluate the effect of salsalate on insulin sensitivity and insulin secretion in subjects with impaired glucose tolerance (IGT) and T2D. We initially investigated the effects of high dose salsalate on glucose and lipid metabolism to facilitate direct comparison to aspirin by achieving a comparable serum salicylate levels in patients with impaired glucose tolerance (IGT) and T2D, then a second cohort at the dose of salsalate recommended in the package insert, 3.0 g/d, in two open label sequential studies. Finally we investigated a mid dose in a double masked placebo controlled parallel design.
Trials 1 and 2 using open label salsalate dosed at 4.5 or 3.0 g/d. Baseline characteristics for study subjects from trials 1 and 2 are presented in Table 7. Since the trials were conducted sequentially, subjects were not randomized and baseline characteristics of the two groups differed. Of note, the seven subjects dosed at 4.5 g/d differed from the nine subjects dosed at 3.0 g/d by having lower fasting glucose and HbA1c and by the inclusion in trial 1 of two persons with IGT.

**Effect of Salsalate on Glucose Metabolism in T2D:** Fasting glucose levels fell by ~20 mg/dl in both the 4.5 g/d and 3.0 g/d cohorts (Figure 8). For the 4.5 g/d trial, this represents a substantial reduction of 19%, from 112 ± 16 to 91 ± 7 mg/dl (P<0.03). Reductions for the 3.0 g/d trial were more modest (9%), falling from 201 ± 23 to 183 ± 20 mg/dl (P<0.05). While one cannot directly compare results between the two trials because the patient characteristics and their overall glycemic control differ, it is important to note that it is more difficult to improve glycemia within the near-normal range (e.g. trial 1, 4.5 g/d) than when gluoses are less well controlled (trial 2, 3.0 g/d). There were no reported symptoms of hypoglycemia or low glucose measurements by periodic home monitoring in either group, despite the overall modest hyperglycemia at baseline in the cohort treated with the higher dose. The glucose lowering effects were not accompanied by changes in body weight in either cohort. (Table 8). Improved glycemia was accompanied increases in fasting insulin levels in both cohorts treated with salsalate, from 44 ± 8 to 67 ± 14 pM (P=0.03) in the subjects receiving 4.5 g/d and from 47 ± 15 to 83 ± 24 pM (P=0.05) in the subjects receiving 3.0 g/d (Figure 8B). This effect could be due to augmented insulin secretion, decreased clearance, or both. However, in the subjects receiving 4.5 g/d salsalate the fasting C-peptide tended to be lower (1.6 ± 0.4 vs. 0.9 ± 0.2 ng/ml, pre vs. post, P=0.06) (Figure 8C), consistent with the diminished insulin clearance demonstrated previously in patients treated with high dose aspirin and during insulin clamp studies described below. Fasting C-peptide concentrations were unchanged in the cohort treated with 3.0 g/d salsalate.

**Effect of Salsalate on Lipids in T2D:** Salsalate therapy was also accompanied by decreases in fasting triglycerides (Figure 8D), 40% in the subjects treated with 4.5 g/d (174 ± 37 vs. 105 ± 20 mg/dl, pre vs. post, P=0.007) and 11% in the subjects treated with 3.0 g/d (150 ± 47 vs. 133 ± 46 mg/dl, pre vs. post, P=0.007). Other changes in fasting lipids were seen only at the higher dose, including reductions in total cholesterol by 12% (201 ± 19 vs. 177 ± 14 mg/dl, pre vs. post, P=0.04) (Figure 8E) and non-esterified free fatty acids (FFA, Figure 8F) by 28% (0.71 ± 0.05 vs. 0.51 ± 0.05 mM, pre vs. post, P=0.02), with small reductions seen in all the major fatty acid subtypes (Figure 9). A reduction in HDL cholesterol (47 ± 3 vs. 43 ± 3 mg/dl, pre vs. post, P=0.01) partially negated the positive effect. However, parallel, but not significant reductions in LDL cholesterol (124 ± 18 vs. 114 ± 13 mg/dl, pre vs. post, P=0.2), resulted in no overall change in the HDL/LDL cholesterol ratio.

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**Table 7: Subject characteristics for the open label 4.5 g/d and 3.0 g/d trials (mean ± SD).** F, female; M, male; BMI, body mass index; FBG, fasting blood glucose

<table>
<thead>
<tr>
<th></th>
<th>Salsalate (4.5 g/d)</th>
<th>Salsalate (3.0 g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>2 IGT/ 5 DM</td>
<td>9 DM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 9</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>32 ± 6</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>112 ± 43</td>
<td>201 ± 23</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.3 ± 1.7</td>
<td>8.1 ± 0.5</td>
</tr>
</tbody>
</table>
We also considered other measures of the metabolic syndrome. Although blood pressure was already well controlled in these patients, there were tendencies towards further reductions in systolic blood pressure at both drug doses and in mean diastolic blood pressure at the 4.5 g/d dose, although these did not reach statistical significance (Table 8). The 8% drop in diastolic pressure in the group receiving 3.0 g/d (77 ± 4 vs. 71 ± 5 mmHg, pre vs. post, P=0.02) was statistically significant. In addition, there was a 16% reduction in ALT (26 ± 4 vs. 22 ± 3 mg/dl, pre vs. post, P=0.01) in the subjects treated with 3.0 g/d; liver enzymes were otherwise unchanged in these small trials (Table 8).

Euglycemic-hyperinsulinemic clamps. In the 4.5 g/d salsalate treatment cohort, glucose infusion rates during euglycemic-hyperinsulinemic clamps increased 44% (P=0.002), with a corresponding 43% improvement in glucose disposal (M) (4.2 ± 0.7 vs. 6.0 ± 0.9 mg/kg/min, pre vs. post, P=0.007) (Figures 10A and 10B).

Indirect calorimetry demonstrated that enhanced glucose utilization was due primarily to improvements in non-oxidative glucose disposal (i.e. glycogen synthesis) (1.9 ± 0.5 vs. 3.2 ± 0.5 mg/kg/min, pre vs. post, P=0.006) without a change in oxidative glucose disposal (2.2 ± 0.2 vs. 2.6 ± 0.4 mg/kg/min, P=0.2) (Figure 10C). Lower fasting glucose levels could not be ascribed to altered endogenous glucose production rates, as this was unchanged at baseline and during hyperinsulinemia. Interestingly, at the higher dose of salsalate resting energy expenditure measured by indirect calorimetry increased 11% (1605 ± 114 vs. 1785 ± 81 kcal/day, pre vs. post, P=0.007), which could contribute to lower fasting glucose levels (data not shown).

Salicylates have previously been shown to reduce insulin clearance [203]. Similarly, insulin levels achieved during the clamp following salsalate administration were higher than at baseline (1.27± 0.11 vs. 2.21 ± 0.23 nM, pre vs. post, P=0.01) (Figure 10E), due to a 40% reduction (P=0.007) in insulin clearance after salsalate (Figure 10D).
Subjects receiving 3.0 g/d salsalate also had significantly improved glucose utilization during euglycemic-hyperinsuline mic clamp analyses, albeit a more modest increment of 15% (3.6 ± 0.8 vs. 4.2 ± 0.8 mg/kg/min, pre vs. post, P=0.003) (Figure 10B). Non-oxidative glucose disposal tended to increase, although this did not reach significance at the lower dose (P=0.06, data not shown). Again, insulin levels achieved during steady state in the clamps post-salsalate were 18% higher (1.32 ± 0.07 vs. 1.56 ± 0.08, pre vs. post, P=0.05) (Figure 10F), which could largely be attributable to a comparable 14% reduction in calculated insulin clearance (P=0.04) (Figure 10D).

### Table 8: Indices for fasting subjects studied in the open label trials before salsalate therapy (Pre) and after 2 weeks treatment (Post) (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Salsalate, 4.5 mg/d</th>
<th></th>
<th>Salsalate, 3.0 mg/d</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>P value</td>
<td>Pre</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.6 ± 8.3</td>
<td>88.4 ± 8.4</td>
<td>0.8</td>
<td>93.7 ± 7.2</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>47 ± 3</td>
<td>43 ± 3</td>
<td>0.01</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>124 ± 18</td>
<td>114 ± 14</td>
<td>0.2</td>
<td>106 ± 11</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135 ± 4</td>
<td>128 ± 5</td>
<td>0.2</td>
<td>135 ± 6</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>75 ± 2</td>
<td>71 ± 4</td>
<td>0.3</td>
<td>77 ± 4</td>
</tr>
<tr>
<td>Anion gap</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
<td>0.08</td>
<td>8 ± 0.2</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>25 ± 3</td>
<td>24 ± 4</td>
<td>0.6</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>21 ± 1</td>
<td>24 ± 2</td>
<td>0.2</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.76 ± 0.06</td>
<td>0.87 ± 0.06</td>
<td>0.005</td>
<td>0.77 ± 0.07</td>
</tr>
</tbody>
</table>

**Figure 9:** Concentrations of individual major fatty acids (mM) are demonstrated before (grey) and after (black) 2 wk 4.5 g/d salsalate. Data represent mean ± SD.
Effect of Salsalate on Insulin Secretion During Intravenous Glucose Tolerance Testing in T2D:
To more fully evaluate the potential role of salsalate in augmenting β-cell function, the acute insulin secretory response was assessed during an intravenous glucose tolerance test (IVGTT). Glucose concentrations were significantly lower in the subjects treated with 4.5 g/d salsalate compared to baseline (Figure 11A) (P<0.01, ANOVA). During the first 10 min of the IVGTT (Figure 11C), insulin concentrations increased 72% in subjects treated with 4.5 g/d salsalate compared to baseline (ΔAUC, 124.5 ± 75 vs. 316.2 ± 145 μU/ml•min, P=0.005). During the same period there was a 50% increase in C-peptide levels (Figure 3E; ΔAUC, 5.7 ± 3.6 vs. 10.6 ± 4.8 ng/ml•min, P=0.04), consistent with this being a true improvement in 1st phase insulin release. Although insulin levels were also higher over the second phase of insulin secretion (10-180 min follow-up period of the IVGTT), C-peptide levels were not increased during this later phase (Figure 11F), suggesting an important contribution of altered insulin clearance, as opposed to enhanced 2nd phase secretion at the later times. While glycemia was marginally lower (ANOVA, P=0.09) following salsalate at 3.0 g/d dosing (Figure 11B), and insulin levels were higher (ANOVA, P<0.001) neither the acute insulin secretory response expressed as change from baseline (ΔAUC), nor the C-peptide response from 0-10 min (Figure 11E) or 10-180 min (Figure 11F) was significantly altered at the lower dose.
**Effect of Salsalate on Inflammation in T2D**: Since salsalate’s primary mechanism of action is anti-inflammatory, we looked at more conventional parameters of inflammation in addition to determining metabolic effects. CRP was reduced ~50% (6.0 ± 2.7 vs. 3.1 ± 1.7 mg/dl, pre vs. post, P<0.05) in subjects treated with salsalate at 4.5 g/d, but was not significantly lowered in the 3.0 g/d cohort (4.5 ± 1.6 vs. 3.3 ± 1.2 mg/dl, pre vs. post, P=0.4) (Figure 8G). Adiponectin also increased 40% in the 4.5 g/d group (9.3 ± 2.1 vs. 13.0 ± 2.5 mg/ml, pre vs. post, P=0.008), and 35% in the 3.0 g/d group (10.7 ± 1.8 vs. 14.4 ± 2.2 mg/ml, pre vs. post, P=0.002) (Figure 8H). Additional markers and mediators of inflammation were assessed at the higher dose where the clinical response was greater. Both interleukin-6 (IL-6) (2.7 ± 0.2 vs. 1.8 ± 0.2 pg/ml, pre vs. post, P=0.1) and white blood cell counts (6.2 ± 0.8 vs. 5.3 ± 0.4%, pre vs. post, P=0.06) tended to decrease, although these changes did not reach statistical significance in this small trial. Like CRP and IL-6, the expression of inducible nitric oxide synthase (iNOS), a major enzyme involved in nitric oxide (NO) synthesis, is a transcriptional target of NF-κB, and targeted disruption of this protein protects against obesity linked insulin resistance [248]. NO is a sufficiently volatile free radical, with a plasma half-life of under 15 s, that its in vivo measurement is difficult. However, it is rapidly metabolized to more stable nitrate (NO3−) and nitrite (NO2−) [281, 282]. Whereas nitrates may be more affected by dietary intake, nitrites can be used as a measure of NO production. Fasting serum nitrites decreased 33% during salsalate therapy (0.49 ± 0.05 vs 0.33 ± 0.04 μM, pre vs. post, P=0.02) (Figure 8I). The decreases in levels of circulating proteins and enzymatic products are consistent with inhibition of NF-κB and further validate this as the target of high dose salicylate.

**Figure 11**. Intravenous glucose tolerance test: A & B, Glucose excursions (mg/dl) and C & D, insulin responses (pM) are demonstrated during IVGTT before (Pre, dashed line) and after 2 wk treatment (Post, solid line) with 4.5 g/d and 3.0 g/d salsalate, respectively. E, The acute (0-10 min) and F, delayed (10-180 min) insulin secretory response to glucose are demonstrated by the area under the curve (AUC) C-peptide, at baseline (Pre, grey boxes) and after 2 wk treatment (Post, black boxes) with 4.5 g/d (left) or 3.0 g/d (right) salsalate. Data represent mean ± SEM; *P=0.04.
To evaluate salsalate effects on NF-κB more directly we looked at its DNA binding capacity in homogenates isolated from circulating monocytes. NF-κB activity decreased after drug treatment by about 65% (P=0.04) (Figure 8I). The effect was reversible and began to return toward baseline levels one wk after discontinuing the drug (data not shown). While it is unknown whether circulating monocytes actively participate in the pathogenesis of insulin resistance, some studies suggest they might [283, 284]. At a minimum circulating monocytes provide a readily accessible source of cells for assessing in vivo NF-κB activity.

**Safety and Tolerability of Salsalate in T2D:** Six of the seven subjects treated with 4.5 g/d salsalate experienced tinnitus, and two required dose reductions due to the tinnitus or headache. These are well-known and expected side effects of high-dose salicylate. Symptoms improved upon dose reduction and all subjects completed the study. Serum salicylate levels were 28.4 ± 2.0 and 19.0 ± 2.0 mg/dl at one wk and two wk of treatment, respectively. Lower levels at the second wk are due, at least in part, to the reduced doses. The salicylate levels seen in the 4.5 g/d trial were comparable to those achieved in the previous study using high-dose (~7 g/d) aspirin (27 mg/dl) [203]. At the standard salsalate dose of 3.0 g/d, no subject experienced tinnitus or required dose reduction, but clinical responses were modest. Serum salicylate levels were lower as well, 5.4 ± 1.3 mg/dl, which would be considered sub-therapeutic by conventional rheumatologic standards.

Only the high dose of salsalate was associated with a tendency towards increased anion gap (Table 8). Both doses were associated with a small but significant increase in serum creatinine (Table 8), although this remained in the normal range and returned to baseline one wk after discontinuation of salsalate. One subject experienced diarrhea at the start of misoprostol that required a dose reduction prior to baseline evaluation.

**Trial 3: Placebo-Controlled Evaluation of Salsalate at Highest Tolerated Dose in T2D.** While our trials demonstrated improved glucose metabolism at both 4.5 and 3.0 g/d doses, the side effect of tinnitus would likely limit clinical applicability at the higher dose, and efficacy was modest at the lower, standard dose. Thus, we sought to determine clinical efficacy in T2D at the maximum tolerable dose. As participation in any clinical trial could alter glycemia by subtle changes in lifestyle, we furthermore employed a placebo-controlled, double-masked design in this third, small clinical trial. This trial was conducted for 4 wk, in contrast to the previous two-week trials utilizing 4.5 or 3.0 g/d dosing, to begin to assess durability, as well. Subjects randomized to the active and treated groups were generally similar at baseline, although cholesterol levels were modestly higher in the placebo-treated group (Table 9).
Five of the eight subjects randomized to treatment tolerated salsalate at the initial 4.0 g/d dose; the remaining three developed mild tinnitus that resolved with reduction to 3.5 g/d. Blood salicylate levels were in the range considered to be therapeutic, 21 ± 4 mg/dl at 2 wk and 13 ± 3 mg/dl at 4 wk treatment. Neither serum creatinine nor anion gap changed in the 4 wk trial, although systolic blood pressure (P=0.01) was 8% higher in the treatment group (Table 9).

Fasting glucose was 18 mg/dl lower (13%) following salsalate (Figure 12A: 136 ± 6 vs. 116 ± 5 mg/dl, pre vs. post, P=0.002), but not placebo (Figure 12B: 127 ± 8 vs. 128 ± 6 mg/dl, pre vs. post, P=0.8). The glucose lowering effect was present at 2 wk and sustained after 4 wk (Figure 12A). The change from baseline in a between group comparison was highly significant (P=0.003) (Figure 12C).

Fasting insulin increased 77% following salsalate (15.6 ± 3.1 vs. 27.8 ± 3.2 μU/ml, P=0.007) and was unchanged by placebo (19.0 ± 2.9 vs. 18.8 ± 2.0 μU/ml, P=0.4). In contrast, fasting C-peptide was reduced 28% following salsalate (4.67 ± 0.74 vs. 3.35 ± 0.62 ng/ml, P=0.002) and likewise, unchanged with placebo (5.59 ± 1.25 vs. 4.45 ± 0.44 ng/ml, P=0.4). As expected, there was no change in homeostasis model assessment of insulin resistance (HOMA-IR) following placebo (5.7 ± 1.0 vs. 6.0 0.7, P=0.5). However, reduced insulin clearance demonstrated during clamp studies in both 4.5 g/d and 3.0 g/d cohorts, and by others following aspirin [203], suggest the 55% increase in insulin resistance (5.2 ± 1.1 vs. 8.1 1.9, P=0.02) calculated using insulin in the HOMA-IR calculation may be misleading (Figure 12D). Using C-peptide rather than insulin in the calculation (HOMA IRC peptide) [285], we demonstrate significant (38%) improvement in insulin resistance in the treatment group (1.53 ± 0.22 vs. 0.95 ± 0.19, P=0.0005) without change in placebo (1.7 ± 0.43 vs. 1.42± 0.17, P=0.4) (Figure 12E). These findings are consistent with the magnitude of improvement in other metabolic parameters found in this cohort, and at this
intermediate salsalate dose lie between the changes in insulin sensitivity demonstrated by euglycemic clamp in the 4.5 g/d and 3.0 g/d groups.

Salsalate also reduced the glycemic response to a mixed meal, with differences in the fasting measurements that were sustained throughout the postprandial period (Figure 12F). Since placebo did not alter fasting or postprandial blood glucose concentrations (Figure 12G), there was a significant difference in ∆AUC glucose values (P=0.006) (Figure 12H). While one month is generally considered to be a very short time for demonstrating changes in glycohemoglobin, a significant reduction was observed in the 4-wk salsalate treatment group (HbA1c: 7.1 ± 0.4 vs. 6.8 ± 0.4, pre vs. post, P=0.03) that was not present in the placebo group (6.7 ± 0.2 vs. 6.5 ± 0.2, pre vs. post, P=0.2).

In the placebo-controlled trial salsalate was also associated with a 33% reduction in fasting free fatty acid concentrations (0.57 ± 0.07 vs. 0.38 ± 0.06, pre vs. post, P=0.0009), which was sustained following a mixed meal challenge (Figure 13A). These metabolic changes were associated with a 66% increase in plasma insulin levels (Figure 5C; ∆AUC, P=0.004) and a 19% reduction in C-peptide concentration (Figure 13E; ∆AUC, P=0.04), consistent with reduced insulin clearance and an improvement in insulin sensitivity leading to a reduction in insulin secretion as assessed by C-peptide concentrations. Plasma glucose, free fatty acids, insulin and C-peptide concentrations were all unchanged from baseline during mixed meal tolerance testing of the placebo group (Figures 12G, 13B, 13D and 13F).

CRP levels did not change significantly in either active or placebo treatment groups. However, adiponectin levels increased 46% in the salsalate group (15.5 ± 1.9 vs. 22.7 ± 2.5 mg/ml, pre vs. post, P=0.001) with no change following placebo (11.2 ± 2.2 vs. 10.6 ± 2.0, pre vs. post, P=0.3). This was accompanied by a highly significant change from baseline in the between group comparison (P<0.0003).

Together, these studies demonstrate the potential benefit of targeting inflammation using salsalate to reduce glycemia, improve glucose utilization, and improve inflammatory measures in patients with T2D. A multicenter study to target inflammation in T2D to reduce glycemia is now underway.
1.3.5.3 Salsalate in Obesity

As we hypothesize that diet induced obesity leads to a chronic subacute inflammation and promotes insulin resistance and the development of T2D, and targeting inflammation in T2D improved glycemia, glucose utilization and improved inflammatory markers and mediators, we assessed the effects of salsalate on glycemia in obese, non-diabetic adults.

**Subject Characteristics**: 20 participants completed the protocol. Subject baseline characteristics were similar (Table 10) and included a female predominance in both groups. Likewise, ethnicity of the two groups was similarly diverse. All participants had normal fasting glucose values. Three subjects (2 in placebo group and 1 in treatment group) had baseline 120 minute OGTT glucose values consistent with impaired glucose tolerance. No subject had diabetes mellitus at baseline.

In treated participants, mean serum salicylate levels were in the established therapeutic range in rheumatology practice (10-30 mg/dL), 18.6 ± 2.5 mg/dL (1.35 ± 0.18 mmol/L) at 2 weeks and 17.1 ± 3.5 mg/dL (1.23 ± 0.25 mmol/L) at 4 weeks. Salicylate levels were undetectable in placebo treated persons. There were no significant changes in weight, systolic or diastolic blood pressure, or standard lipid profiles in either group.

**Effect of Salsalate on Glucose Metabolism in Obesity:**

The change in fasting glucose from baseline between salsalate and placebo groups was highly significant after one month: with a reduction of 8 ± 4 % following salsalate, compared to an increase of 5 ± 2 % following placebo (p<0.002) (Figure 14a, left). Likewise, glucose area under the curve during oral glucose tolerance testing was reduced by 14 ± 5 % after salsalate and increased by 6 ± 3 % following placebo for a highly significant improvement from baseline in between group analysis (p= 0.003) (Figure 14a, middle). In addition, the glycated albumin was reduced by 17 ± 4% salsalate compared to 0.6 ± 0.9% after placebo (p < 0.002) (Figure 14a, right). Thus in between group analysis, multiple measures of glycemia improved in salsalate compared to placebo treated groups. Within group comparisons also demonstrated improvement following salsalate, but not placebo. The glycemic response to glucose load improved following salsalate treatment (repeated measure analysis p<0.01), but not placebo during the one-month study (Figure 14b). Within groups, one month of salsalate resulted in a significant reduction in fasting plasma glucose level from 90.8 ± 2.7 mg/dL (p<0.03), whereas subjects receiving placebo had a non-significant increase in fasting plasma glucose from 86.9 ± 1.8 vs. 91.1 ± 2.1 (p=0.1). Finally, glycated albumin decreased following salsalate from 12.4 ± 0.4% to 10.3 ± 0.3% (p<0.003) but was unchanged following placebo 12.5 ± 0.3% to 12.4 ± 0.4% (p=0.5). Therefore, glycemia, assessed by fasting, post-glucose load

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**Table 10: Baseline Subject Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Salsalate Therapy</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1M/8F</td>
<td>2M/9F</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>5W/3H/1B</td>
<td>5W/1H/4B/1other</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.5 ± 1.1</td>
<td>24.1 ± 1.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>36.3 ± 2.2</td>
<td>38.9 ± 2.5</td>
</tr>
<tr>
<td>Current smoking</td>
<td>3 of 9</td>
<td>2 of 11</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
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<td>4.6 ± 0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
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<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Systolic BP</td>
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<td>123 ± 4</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>67 ± 3</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>6.7 ± 0.4</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>120 minute glucose (mmol/L)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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and glycated albumin, was significantly reduced following salsalate when compared to a placebo treated group. No symptomatic hypoglycemia was noted in either group.

Fasting insulin values were unchanged in both groups. Similarly, insulin levels following glucose challenge remained unchanged pre- and post-salsalate and placebo exposure (Figure 15a).

However, the change in fasting C-peptide between salsalate and placebo treated groups was highly significant, with a reduction of 30 ± 7 % following salsalate and increase of 5 ± 6 % following placebo (p<0.01). Within groups, fasting C-peptide levels decreased following salsalate from 5.0 ± 0.7 vs. 3.4 ± 0.5 ng/mL (p=0.02), but remained unchanged following placebo from 4.5 ± 0.5 vs. 4.6 ± 0.5 ng/mL (p=0.5). The change in C-peptide AUC post glucose challenge was also significantly different between groups with a reduction of 23 ± 4 % following salsalate and increase of 4 ± 7 % following placebo (p=0.02) (Figure 15b and 15c, left).

Salicylates have previously been demonstrated to reduce insulin clearance[203]. Taken together, these data suggest improved insulin sensitivity as further supported by homeostasis model assessment of insulin resistance calculated using C-peptide rather than insulin (HOMA IRc peptide), which was significantly lowered by 35 ± 8 % in the treatment group and increased 9 ± 5 % following placebo (p =0.003) (Figure 15c, right). However, HOMA-IR using insulin was not significantly changed in either group: salsalate 4.6 ± 0.9 vs. 4.2 ± 0.6 (p=0.9) and placebo 3.5 ± 0.5 vs. 3.5 ± 0.5 (p=0.7), pre vs. post treatment, respectively, with no difference in the
change between groups (p=0.7). These findings are consistent with reduced insulin clearance and improved insulin sensitivity contributing to the improved glycemia.

**Effect of Salsalate on Inflammation, Cytokines and Adipokines, in Obesity:**

To evaluate the proposed anti-inflammatory mechanism of action for improvement in glycemia, cytokines and adipokines were assessed pre- and post-one month of therapy. Importantly, inflammatory markers also showed significant improvement following salsalate when compared to the placebo treated group. Adiponectin increased 56 ± 17% in the treatment group and decreased 1 ± 5% in the placebo group (p=0.003), consistent with improved insulin sensitivity (Figure 16a).

In the within group analysis, adiponectin showed a significant increase in the treatment group (10.6 ± 1.7 vs. 16.2 ± 2.7 mg/L, p=0.01), and was unchanged with placebo (10.8 ± 1.5 vs. 10.5 ± 1.5 mg/L, p=0.5).

In change from baseline analysis, CRP concentrations decreased from 5.1 ± 1.1 vs. 3.2 ± 0.8 mg/L (p=0.05) following salsalate, without a significant change

**Figure 15:** Insulin, C-peptide and Insulin sensitivity

15a and 15b: Mean and standard error data are demonstrated before and 30, 60, 90 and 120 min following 75 g oral glucose for insulin (15a) and C-peptide (15b). Baseline data is depicted by the dashed line and open circle, and post treatment data by the solid line and closed circle.

15c: The C peptide response to Oral Glucose Tolerance Test calculated by AUC showed a significant between group difference. Insulin sensitivity calculated by HOMA IR C-peptide showed a significant between group difference with improvement in the salsalate compared to the placebo treated group. Black bar = salsalate, Grey bar = placebo.
following placebo from 4.8 ± 0.9 vs. 4.5 ± 1.0 mg/L (p=0.7) (Figure 16b, left), and fasting non-esterified fatty acids (FFA) levels showed a decline following salsalate from 0.46 ± 0.08 to 0.25 ± 0.05 mEq/L (p=0.05) with no change following placebo, from 0.45 ± 0.07 to 0.39 ± 0.09 mEq/L (p=0.6) (Figure 16b, right), although the between group comparison did not reach statistical significance. Additional inflammatory markers IL-6 and soluble VCAM-1 did not change significantly following salsalate therapy (data not shown).

**Safety and Tolerability of Salsalate in Obesity:**

All participants were initiated on 4.0 grams/day of salsalate or placebo given orally, divided in two equal doses. Three participants required dose reduction due to complaints of tinnitus, headache or dizziness. Two of the three participants were on study medication while one was on placebo. All participants completed the trial on the reduced dose. Of the actively treated participants, one tolerated 3.5 grams per day and one tolerated 3.0 grams per day without symptoms.

Salicylates are known to cause anion gap acidosis and NSAIDs as a medication class have been associated with alterations in renal function. There were no noted changes in laboratory analysis of renal function, electrolytes, or anion gap during this trial. Although salsalate has a rare reported prevalence of allergic reactions [286, 287], three participants who received active therapy were withdrawn due to a rash that developed while on therapy. No respiratory distress was noted. Although there was no statistically significant change in mean alanine aminotransferase (ALT) and aspartate aminotransferase (AST) over the one-month period in the treatment or placebo groups (p>0.1), two actively treated participants were noted to have transient mild transaminitis at the final visit. One participant had an isolated rise in ALT to less than twice the upper range of normal, and a second participant had a similarly mild elevation in both ALT and AST. Both resolved spontaneously. Obesity is associated with mild elevations in serum AST and ALT, often associated with hepatic steatosis and/or hepatic inflammation. The true prevalence of this
problem is unknown, as the majority of affected individuals are asymptomatic. However, recent estimates suggest over 33% of obese individuals may have hepatic steatosis [288, 289].

Therefore, the presence of mild elevations in hepatic enzymes noted in our study may be consistent with the baseline in the obese population studied. Salsalate has been widely prescribed for more than a half century and no specific concerns in overweight persons have been noted. Safety will need to be evaluated in the proposed studies.

**Summary:** These studies demonstrate that salsalate lowers glycemia, and reduces inflammatory markers and mediators that have been demonstrated to participate in the development of diabetes and the atherosclerotic process. Further studies are now warranted to demonstrate the safety and efficacy in patients with the metabolic syndrome and CVD.

**In conclusion:** These preliminary investigations in patients with T2D and/or obesity provide the groundwork to begin new investigations to determine the effects of salsalate at maximum tolerable dose in patients with metabolic syndrome and stable CVD to evaluate the therapeutic potential of IKK/NF-κB inhibition in the treatment of these syndromes. Studies have been initiated to evaluate the role of targeting inflammation in patients with T2D to lower blood sugars.

The studies proposed in this grant will also provide new information on the molecular mechanisms of action of salicylates as potential therapeutic agents in the treatment of these highly prevalent disorders. These findings will further test hypotheses relating inflammation to the pathogenesis of insulin resistance, metabolic syndrome and CVD and IKKβ as a novel target for the reversal of these disorders.

**1.3.6 Validation of IKKβ and NF-κB as targets for reversal of insulin resistance**

We have conducted numerous studies in order to validate IKKβ and NF-κB as pharmacological targets for the reversal of insulin resistance and to identify the primary tissues that are involved. These studies conducted primarily with transgenic and knockout mouse models firmly support the original hypothesis and identify fat and liver as primary sites of involvement. By contrast, muscle and β-cells appear to be secondary targets that are affected by cytokines produced in fat and/or liver.
Diet-induced obesity: NF-κB activation and gene induction in Fat and Liver. To begin testing potential roles of IKKβ/NF-κB in acquired insulin resistance, fat and liver were ice fed a high-fat diet for 8 weeks. Diet induced obesity and insulin resistance were appropriately confirmed, with increased body weight due to increased adiposity (dexam scanning), increased circulating leptin levels consistent with increased adiposity, and insulin levels consistent with the development of insulin resistance. EMSA assays conducted with harvested tissues indicated that NF-κB was activated (translocated into the nucleus) in fat (Figure 17). Results from quantitative RT-PCR analyses supported this. NF-κB target genes whose expression was enhanced included IKKβ, the IKK scaffolding protein IKKβ, TNF-α, and PAI-1. Also elevated were mRNA levels for STAT and SOCS proteins (Figure 17), potentially due to activation by additional cytokines that are targets of NF-κB (e.g. IL-6, IFN-γ). IKKβ and NF-κB were similarly elevated in liver of high fat fed mice (Figure 17).

LIKK: NF-κB activation in liver. Low-level transgenic expression in liver of constitutively active IKKβ SS/EE was successfully achieved, to match that seen in diet-induced obesity (Figure 18). LIKK mice are viable and appear normal, they eat normal amounts of food and weigh the same as their wt littermates. Organs and tissues, including liver, have normal weights and histological appearances. There is no gross evidence of infiltrating immune or inflammatory cells, and architecture is normal. Circulating liver transaminases levels are normal.

Insulin resistance in LIKK mice. Fasting and post-prandial insulin and glucose levels are elevated in LIKK mice. HOMA-IR (fasting glucose x fasting insulin) is an excellent correlate for insulin sensitivity [290]. HOMA was elevated in LIKK mice (4.2 ± 0.4) relative to WT littermates (3.2 ± 0.3; p<0.01). Insulin resistance in LIKK mice is dose dependent, as homozygous Tg+/+ mice carrying two copies of the transgene...
are more insulin resistant than hemizygous Tg+/− mice (Figure 19), and both sexes are affected. Hyperinsulinemic-euglycemic clamps confirmed profound insulin resistance in liver and significant resistance peripherally as well, suggesting a potential humoral effect.

**A humoral mediator.** Numerous methods were used to identify potential mediators, including microarrays and assays of circulating cytokines. Microarray and confirmatory RT-PCR results pointed to IL-6 and IL-1β. Message levels for these pro-inflammatory cytokines were elevated in liver by DIO (6.1- and 3.5-fold, respectively) and in LIKK liver (8.8- and 3.7-fold, respectively), relative to chow-fed WT littermates.

Neutralization experiments determined whether IL-6 was actually mediating insulin resistance in LIKK mice. Anti-IL-6 antibodies for neutralization purposes are commercially available for treating men or mice. Following standard protocols, we treated FIKK mice with 120 mg of the antibody each (n=4), and ten days later analyzed insulin resistance using the HOMA-IR method (fasting glucose x fasting insulin). Insulin resistance reversed, suggesting that in LIKK mice IL-6 plays a role in insulin resistance (figure 20). Findings with LIKK mice have been recently published in Nature Medicine [206] but are included in the addendum materials. Notably, we have used similar approaches to neutralize IL-6 in the circulations of DIO and ob/ob mice with equally significant results, suggesting again that IL-6 plays a significant role in the development of insulin resistance in these models.

**LISR: NF-κB inhibition in liver.** LISR mice, selectively expressing the IκBα super-repressor in liver, are viable and normal appearing. LISR and WT littermate body weights, food intake and liver histology are indistinguishable (data not shown). LIKK and LISR mice were crossed to determine whether the insulin resistance phenotype was dependent on NF-κB activation. Co-expression of the IκB super-repressor in LIKK x LISR mice reduced both insulin (AUC: LIKK, 57.7 ± 5.5 vs. LIKK x LISR, 43.0 ± 7.9 ng/ml•h; P=0.05) and glucose (AUC: LIKK, 323 ± 16.2 vs. LIKK x LISR, 275 ± 12.2 mg/dl•min; P=0.04) concentrations during glucose tolerance tests, which translated into marked improvements in insulin resistance index (Figure 21). These findings verify that the development of insulin resistance in LIKK mice is NF-κB dependent, as opposed to being mediated by IKKβ activation through an alternative pathway, and supports the hypothesis that IKKβ/NF-κB inhibition provides a viable target for sensitization.

LISR mice were used further to test whether liver-specific inhibition of NF-κB protected against the development of diet-induced insulin resistance. LISR mice and WT littermates, fed a HFD for 3 months, gained similar amounts of weight on the diet. Both insulin (AUC: WT, 92.8 9.5 vs. LISR, 63.9 8.5 ng/ml•h; P=0.04) and glucose (AUC: WT, 352 16 vs. LISR, 289 16 mg/dl•min; P=0.03) concentrations were lower in the LISR mice during glucose tolerance testing. Insulin resistance index was accordingly decreased in LISR mice relative to wt littermates (Figure 21). These latter findings indicate that NF-κB activation in
Liver parenchyma is an integral feature of insulin resistance associated with diet-induced obesity.

**Insulin resistance in FIKK mice.** Transgenic low-level expression of constitutively active IKKβ SS/EE was successfully achieved in fat as well. IKK and NF-κB were elevated ~1.5 fold over WT. FIKK mice are viable and appear normal. Organs and tissues other than fat have normal weights and histological appearances. There is no evidence of infiltrating immune or inflammatory cells in the fat. Nevertheless, fat pad weights are elevated by 46%, without an increase in cell number, due to a 47% increase in adipocyte size.

**Insulin resistance in FIKK fat and FIKK mice.** We first showed that FIKK fat is insulin resistant in terms of insulin stimulated glucose uptake in vivo as well as in isolated fat cells (ex vivo). Even more importantly, FIKK mice have systemic insulin resistance. Glucose tolerance testing (GTT) of the mice revealed elevations in both glucose and insulin levels, and this was again dose dependent -- compare the results for the typical hemizygous transgenic FIKK1 mice with results from homozygous FIKK2 mice expressing two copies of the transgene (Figure 22). Because direct effects such as glucose uptake into fat alone are unlikely to be sufficient to cause systemic insulin resistance, these data suggest that there might be cross-talk between fat and other tissues.

Hyperinsulinemic-euglycemic clamps were used to assess the degree of systemic insulin resistance and to begin to identify which other tissues might be affected in FIKK mice. Glucose infusion rates and glucose disposal were significantly reduced in FIKK mice relative to wt littermates, suggesting possible involvement of muscle -- hence cross-talk between fat and muscle (humoral or neural) or a paracrine effect of fat in muscle. Insulin’s ability to suppress hepatic glucose production (HGP) was similarly suppressed ~40% in wt mice and ~25% in FIKK, suggesting a possible effects on liver as well. Effects in muscle were looked at directly using a glycogen synthesis assay, which revealed a clear impairment in FIKK mice. Taken together these findings strongly support the notion that activating the pro-inflammatory NF-κB pathway in fat, a non-classical inflammatory tissue, leads to systemic insulin resistance through direct effects on fat as well as through distant effects in other tissues.

**Fat transplant.** To directly examine the ability of fat to induce insulin resistance, and determine whether it is a humoral, neural or paracrine effect, equivalent amounts of fat tissue from WT and FIKK donors were transplanted into genetically identical, WT recipient littermates following Reitman’s protocols. Recipients of FIKK fat were more insulin resistant, with higher fasting and post-glucose challenge blood glucose and insulin levels, than recipients of WT fat. These findings clearly point to humoral effects (Figure 23).

**FISR: NF-κB inhibition in fat.** FISR mice, selectively expressing the IκBα super-repressor in fat, are viable and normal appearing. We had a predicted protection against the development of insulin resistance, and it appears that we have gotten this and more. There are dramatic reductions in both fasting and post-glucose challenge blood glucose and insulin levels in both chow and high fat fed mice.

While we had not predicted an effect on body weight, there are dramatic decreases in body weights of both high-fat fed mice and ob/ob mice crossed with FISR. Ob/+ and FISR mice were crossed to generate FISRxOb/+ mice, which were crossed with Ob/+ mice to generate ob/ob x FISR mice. Fasting and post glucose challenge blood glucose levels are markedly reduced in ob/ob x FISR mice compared to ob/ob mice (Figure 24). Body weights were significantly lower in ob/ob x FISR mice compared to ob/ob mice. On a high fat diet FISR mice tend not to gain, and weigh about the same as wt mice on normal chow. Thus wt mice on a high fat diet weighed ~20% more than wt mice on chow, and FISR mice on HFD weighed ~20% less than wt mice on
HFD (Figure 25). These findings are particularly interesting in light of our discovery of an increase in basal metabolic rate in humans treated with high dose salicylate.

In conclusion, activation of NF-κB in fat or liver leads to insulin resistance. Inhibition of NF-κB in fat or liver does the converse, it protects against the development of insulin resistance, and in the case of fat, against obesity as well. Similar but preliminary studies looking at the effects of activated or inhibited NF-κB in muscle or β-cells indicate that these are not primary sites of NF-κB mediated insulin resistance. Given the dramatic role of NF-κB on mediators of inflammation, obesity, insulin resistance, all central to the pathogenesis of atherosclerotic disease, we seek to determine the role of inhibition of NF-κB signaling in vascular remodeling in humans.
CHAPTER 2: DESCRIPTION OF RESEARCH PROTOCOL

The study is a multi-center, prospective, interventional design. There are two trials: one randomizing patients to intensive lifestyle intervention or usual care and the second randomizing patients to salsalate or salsalate placebo. It is recognized there is no true masking for participation in a diet and exercise program, however the salsalate trial will be double masked and both groups will be compared to their respective usual care or placebo. Therefore, the protocol will first be presented for both trials jointly and afterwards the lifestyle trial and the salsalate trial will be described separately in more details.

2.1 Study Design

The study is a trial of overweight/obese patients with known stable coronary heart disease. There are two separate trials: one with lifestyle modification and Lovaza, a prescription medication available in the US only with a physician’s prescription, and the second with IKKβ/NF-κB inhibition using salsalate. Since the exclusion criteria are stricter for salsalate trial, those patients potentially eligible for salsalate will be screened for salsalate and if eligible will be randomized to salsalate or placebo. If they are not eligible for salsalate or decline the salsalate study, they will be offered the lifestyle study. Patients not eligible for salsalate but eligible for lifestyle will be randomized to the lifestyle intervention or usual care.

This is a 30-month study with a 4 month screening period and a 30-month treatment period. While the lifestyle arm and its usual care control cannot be blinded, the salsalate and the placebo arms will be double-blinded. There will be a total of 7 visits (with 3 additional phone and blood test visits for the salsalate trial). The baseline and final evaluation will be performed at the General Clinical Research Center (GCRC) of the Beth Israel Deaconess Medical Center (BIDMC)/Joslin Diabetes Center (JDC). Interim visits will occur in the office of the patient’s cardiologist care provider, or at BIDMC/JDC. Approximately 340 patients will be screened for the lifestyle trial and 390 for the salsalate trial. With an estimated 18% screen and baseline failure rate for lifestyle, 278 will be randomized in lifestyle with 139 to active dietary, exercise and nutritional supplementation (with omega-3 fatty acids in the form of Omacor (now known as Lovaza), a prescription medication available in the US only with a physician’s prescription), and 139 to usual care in the lifestyle trial. With an estimated 30% screen and baseline failure rate for salsalate, 278 will be randomized in the salsalate trial: 139 to salsalate and 139 to salsalate active

Table 12: Trial Schedule

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<th>Visit Number</th>
<th>Screening</th>
<th>Baseline</th>
<th>Active treatment period</th>
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</thead>
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<td>x x</td>
</tr>
<tr>
<td>CT angiography (BIDMC)</td>
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</table>

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For all participants, there will be about a 4 month screening period, pre-treatment baseline evaluation and a 30-month treatment period followed by an end of treatment evaluation. The baseline and end of treatment evaluations will consist of medical history, physical exam and fasting laboratory measures and assessment of calcified and soft plaque in the coronary arteries by Multi-detector computed tomographic angiography.

The trial schedule is shown in Table 12 (Visits 3, 4 and 6 for salsalate and its placebo arms only). Additionally, note that in the lifestyle trial, phlebotomy and Quest Laboratory analysis are at Screening, Baseline, Month 12, months 24 and 30 only.

The central laboratory is the Lipid Metabolism Laboratory of the Jean Mayer USDA Human Nutrition Research Center at Tufts University, run by Dr. Ernst Schaefer and Bela Asztalos, PhD. Quest laboratories will be running the routine laboratory assays.

**Study subject** full inclusion and exclusion criteria are provided below in the application (sections 4.1 and 4.2). In brief:

Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (≥6 months ago), previous coronary artery bypass surgery (>12 months ago), stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery or abnormal exercise tolerance test. Subjects should be at list 6 months after a myocardial infarction or abnormal exercise tolerance test.

Subjects who have had coronary artery bypass grafting less than 12 months ago or unstable angina are not eligible. Subjects must be on a statin or unable to tolerate a statin.

Subjects must also have either a BMI of ≥ 27 kg/m² (26.6 is rounded to 27) as a surrogate of the metabolic syndrome or a BMI of 25 to 26.5 with an elevated C-reactive protein or with increased waist. Assessment of increased waist will be determined based on the criteria described in “Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and international association for the Study of Obesity” guidelines, or for lifestyle with 2 additional components of metabolic syndrome. (to prevent under-representation of minority persons in our investigations, we will use a BMI ≥24.5 for subjects from Asian origin [291])

In addition, subjects must be: a) aged 21-75 years inclusive; b) have BMI ≤ 35 kg/m² if females and ≤ 40 kg/m² if males; c) on a stable dose of an HMG CoA reductase inhibitor (statin) for 1 month at screening or unable to tolerate a statin; d) have normal renal function (note estimated creatinine clearance (eCrCL) calculated using Cockcroft-Gault (CG) [292] equation ≥60 at screening [eCrCLCG (ml/min) = [(140 – age) x weight (kg)]/[SCr (mg/dl) x 72] x [0.85 if female] or allowing inclusion of subjects with a serum creatinine of <1.3 if CrCl is < 60 ml/min/1.73m² and there is no history of renal disease; e) have liver function (ALT, AST) < 3 times upper limits of normal; f) normal thyroid function (on stable dose replacement therapy is acceptable); g) if women are of child bearing potential they must have a pregnancy test prior to the CT angiography and agree to use contraceptive methods for the duration of the study; h) For those entering lifestyle, they must have an Exercise tolerance test (ETT) performed within 12 months provided there has been no increase in patient’s anginal pattern. (If there has been a change in their anginal pattern over the 12 months before the study, study subjects will need a new ETT). and i) For the salsalate trial patients with T2D must have a fasting glucose of ≤ 200 mg/dl at screening and cannot be treated with thiazolidinedione class agents or insulin or Exendin-4 (Byetta) therapy.
(Full exclusion criteria are provided below in the application, section 4.2).

**Concomitant Medications.** Any medications administered during the study period will be documented. Prescribed medications for co-morbid conditions including hypertension will be permitted, if the patient/subject has been on a stable dose for at least 30 days. Good medical practice may necessitate altering medications for co-morbid conditions if absolutely necessary. Patients cannot participate in any other investigational drug study while participating in this study.

**Recruitment and Screening of Patients at BIDMC/JDC/Clinical Cardiology Practice Sites.** Patients with CVD will be recruited through the Beth Israel Deaconess Medical Center (BIDMC) and the Joslin Diabetes Center (JDC) clinic as well as recruitment using posters, ads in the Metro and radio advertisements. In addition, cardiology practice sites participating in this research project will participate in patients’ recruitment. Interaction with the sites will be coordinated by the study coordinators for lifestyle (for those patients entering lifestyle) and the study coordinator for salsalate (for those entering salsalate). Human Investigation Review Board Approval will be through the BIDMC, Tufts University, as well as the New England Investigation Review Board (NEIRB - 40 Washington Street, Suite 130, Wellesley, MA 02481, Phone: 781-431-7577). Cardiology practice sites participating in this study are in the greater Boston area.

Several cardiology practice groups and the BIDMC cardiology clinic and JDC clinic will participate in the recruitment.

Cardiology sites may require centralized IRB from the New England Institutional Review Board (NEIRB), Wellesley, MA. BIDMC approved consent documents will be provided to the centralized IRB to maintain uniformity. However, any subject consented offsite will be re-consented by a study team member at the time of the first visit to the BIDMC/JDC.

Each cardiology site will interact directly with the study coordinator, nurse and physician such that scheduling follow-up visits and scheduling and monitoring of laboratory values and adverse events will be performed by the study coordinator. However, the nurse or study coordinator at each clinical site will have to fill out information at the 6, 12, 18, and 24 month visits, and provide this information directly to the study administrative core office at BIDMC/JDC.

**Visit Schedule**

**Screening Visit (Visit 1):** The Screening visit will occur between -4 months and –1 week at BIDMC/JDC/Cardiology sites. After explaining the nature of this study in detail, written informed consent will be obtained before any study procedure being performed. Subjects will be screened for inclusion and exclusion criteria. The screening visit will include a medical history, physical exam, height, weight, waist circumference measurements, vital signs, screening lab panel (fasting glucose, Chem profile, CBC, Lipid panel, TSH and urine microalbumin/creatinine) and rectal exam for stool guaiac. (Latter for salsalate trial only.) If the subject has had a colonoscopy (negative for CA) in the last 3 years, no stool guaiac/digital rectal exam (DRE) will be required. At this time, we will obtain an EKG performed within one year from the patient’s provider.

If a baseline EKG cannot be obtained at screening, the EKG performed at visit 2 will be used to establish a baseline.

CVD patients would be scheduled for a fasting screening at a local Quest site after being consented at the cardiologist’s office. The cardiology office would notify the administrative core study coordinator in the central Vascular Remodeling Study (VRS) office at BIDMC about the
subject’s name, address and contact information. All this information will be kept confidential and only the study nurses, study coordinator and the principal investigators will have access to it. The administrative core study coordinator will obtain blood test results and be responsible for consent forms. The patient would then have his/her blood drawn at their cardiology office and sent to Quest or go to the nearest Quest site in New England near their home for the screening, and the results will be sent to the VRS office using a Quest form with the VRS account number.

A participant who is found to be ineligible during screening may be re-screened once within 3 months. If ineligible on the second screening for the same reason, the participant is permanently ineligible.

If a health issue is identified on the initial screen that leads to altered drug therapy, the participant may be re-screened, after establishing a new stable baseline.

**Enrollment (Baseline) Visit at BIDMC/JDC**

Approximately 626 patients will undergo baseline evaluation (about 556 total are expected to remain in the study after initial MDCTA). After explaining the nature of this study in detail, written informed consent will be obtained again for subjects who had their screening evaluation at outside cardiology sites. Despite the fact that the study will have been described and consent obtained by the cardiology site physicians to ensure the quality of the consent procedure, we will repeat consent by a member of the BIDMC/JDC study team. Subjects will then have a fasting blood test, medical history, vital signs, physical examination including height, weight, waist circumference measurements, questionnaires, electrocardiogram and baseline MDCTA. Blood will be drawn for fasting glucose, HbA1c, insulin and c-peptide. Among consenting participants, blood samples and immortalized lymphocytes will also be obtained and stored for DNA, mononuclear lymphocyte RNA for gene expression. Serum/plasma will be stored for protein and lipid fraction analysis at a later date, a urine sample will be collected and electrocardiogram performed. Full details are provided in the baseline and end of treatment section (see below).

Patients qualifying for the study by the results of their screening visit MDCTA will be randomized to the active or usual care or placebo arm in either the lifestyle or salsalate trial. It is anticipated that 626 patients will undergo baseline visit and MDCTA and 556 patients will remain after MDCTA failure: 278 in lifestyle and 278 in salsalate. All subjects will be counseled to take Aspirin 81-325 mg daily, be physically active, and smokers will be advised to cease. Subjects will continue to take all other concomitant medications at the same time taken before the start of this study.

Once a patient is assigned to a specific study trial the patient is then followed by that study nurse or coordinator and that study principal investigator (PI) for the life of the study. Patients assigned to other than lifestyle intervention or usual care will not know to which arm of the study they have been assigned. The PI will know whether they are getting active lifestyle intervention or usual care but not whether they are receiving salsalate or placebo.

**2.2 Specific Protocol to Lifestyle Intervention**

The Jean Mayer USDA Human Nutrition Center on Aging at Tufts University will provide the dietary counseling for the lifestyle intervention. The lifestyle intervention by definition will be more labor intensive and is modeled on the Diabetes Prevention Project. This Exercise program will be implemented by the lifestyle managers Dr.’s Francine Welty and Ernst Schaefer, and a study coordinator, and nutrition by a study dietitian who will be working under the supervision of Dr. Schaefer to implement the dietary intervention. Subjects will elect to have either frequent telephone or email contact with both the lifestyle coordinator for exercise of at
least 5 hours per week of aerobic exercise and strength training, and also for instruction with the dietitian. We have redesigned the web-based 12 session core curriculum of the Diabetes Prevention Project (http://www.bsc.gwu.edu/dpp/index.htmlvdoc) to achieve the following reverse pyramid:

Five—or more hours of moderately vigorous exercise weekly including aerobic exercise and strength training, with the advice and support of their cardiologist.

Four—or more servings of non-starchy vegetables and fruits to include a variety of fresh, frozen, or canned vegetables and fruits that are low in glycemic index or load. Dried fruits are not included in this category. Avocados should be limited to one fruit daily. Vegetables should comprise at least half of the total vegetable and fruit intake with 4 or more cups of various non-starchy vegetables and fruits daily.

Three—or more 8 ounce servings of various healthy protein sources including fish, poultry, meat, egg whites, beans, soy protein, and non or low fat dairy products. Protein selections should be rich in calcium to maintain optimum bone health. Poultry and meat should be as lean as possible, preferably at least 95% lean. Free-range, wild, or grass-fed animal sources are preferable to grain-fed animal sources.

Two—two cups of full-fiber whole grains or starchy vegetables daily: full-fiber whole grains, such as rice, oats, barley, wheat, corn, quinoa, and other grains are recommended, provided that grains have not had the fiber removed during processing. Choose starchy vegetables that are high in fiber. Starchy foods can be replaced with vegetables and fruits if desired.

One—one ounce of healthy fats from oils and nuts daily. Aim for 1 ounce daily from unsaturated vegetable oils and nuts or nut butters. Oils high in omega-3 fatty acids and omega-6 fatty acids such as canola oil or soybean oil are recommended. Animal fats and dairy fats should be minimized and hydrogenated or partially hydrogenated trans fats should be avoided if possible. Vegetarian sources of saturated fat such as tropical oils should be avoided.

Zero—added sugar and unhealthy trans fats; added sugars are those in beverages, processed foods, condiments and baked goods and should be avoided. Fruit juices and dried fruits should be consumed in small quantities or avoided due to high sugar content.

Study subjects in the active lifestyle group will be provided with the book "A Healthier You," (339 pages) published in 2005 by HHS (which costs $9.00 from Amazon.com) and follows the 2005 US Dietary recommendations which we will modify to decrease the glycemic index, lower saturated fat to < 7%, cholesterol to < 200 mg/day, and simple refined sugars to < 10% of calories as recommended by WHO. Our aim is to get people to a BMI of 25 or less over 30 months. Caloric restriction will be by 500-1000 calories per day, and the exercise component will be to get people into the active category (walking 3 or more miles per day at 3-4 miles/hour - i.e. around 1 hour either outside or on a treadmill or using an exercise bicycle for 1 hour per day. We will prefer to have people break it up into two 30 minute segments per day and if indoors they then watch one full cycle of CNN Headline news in the AM and the PM at their health club, or at home on their treadmill or exercise bicycle. Pedometers will be used to quantify exercise amount. Calorie assessment will be done on exercise bicycle or treadmill. The study nutritionist, will be giving groups of 10, a total of 12 telephone classes weekly (a total of 30 groups), and then monthly telephone follow-up with the group for people to share their progress. Our prior experience with groups has been very favorable. We will be using a telephone conferencing service. Study subjects in the active lifestyle group will be prescribed four Lovaza (omega 3) capsules, which will be shipped directly to study subjects from Pharmacy Benefits Management (PBM) Plus, Inc. Subjects will be recommended to use two servings (2 tablespoons) per day of full fat high alpha linolenic acid Promise margarine from Unilever (which
also contains quite a bit of linoleic acid and plant sterols-and will ensure adequate intakes of essential fatty acids).

2.2.1 Exercise

Study subjects will have a graded exercise or pharmacological stress test within 12 months prior to visit 2 provided there has been no change in anginal pattern during that time. If they have had a change in anginal pattern, they need a new exercise tolerance test (ETT). Dr. Welty will review all ETT results, calculate maximal target heart rate and provide exercise prescriptions for each study subject based on their ETT results. We will provide instructions that they not exceed 70-85% of their predicted maximal heart rate if they have no angina. If they have stable angina, they must not exceed 70% of the heart rate at which they developed angina during their stress test. For patients without angina during stress and with a reversible defect on nuclear imaging or an inducible wall motion abnormality during echocardiography without subsequent revascularization, exercise will be limited to 70% of the peak HR achieved during exercise. We will instruct subjects how to take pulse for 15 seconds and multiply by 4. Hydration will be recommended before exercise. We will also recommend an ETT at the end of the study to the referring physician. We now plan to capture this fitness data in the database.

For those who currently exercise, they will continue their current program. We will have them start exercise at their current level and increase by 5 minutes each week with our goal being 60 minutes daily. This can be in divided sessions throughout the day.

For non-exercisers, we will recommend a brisk walking program, swimming, elliptical machines, stationery exercise bike or rowing machine and increase exercise by 5 minutes weekly to the goal of 60 minutes daily. Exercising with a partner or significant other will be recommended if study subjects are walking or doing exercise other than swimming or in a health club.

We will provide standardized educational materials and instructions and algorithms regarding the development of symptomatic chest pain or other problems that arise secondary to the exercise component in this protocol (see instruction manual).

In addition to the algorithms, we plan to call study subjects weekly to inquire about new symptoms during exercise. We will provide a calendar for study subjects to record their daily amount of exercise and note any new symptoms that may occur during exercise (see attached calendar). They will be specifically asked about chest pain, chest pressure, abdominal pain, shortness of breath, dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea, palpitations or claudication. If they have chest pain or pressure or an anginal equivalent, we will ask about nausea, vomiting, radiation of the pain/pressure or diaphoresis.

If they develop adverse events (unstable angina or other CVD events in association with exercise), they will need to be treated appropriately by their treating physicians. If they have a repeat ETT, which is acceptable and once their MD feels they can resume exercise, they can resume the program. They will not exit the study.

2.2.2 Visit schedule

Screening Visit: The Screening visit will occur between -4 months and –1 week at BIDMC/JDC/cardiology sites. After explaining the nature of this study in detail, written informed consent will be obtained before any study procedure being performed. Subjects will be screened for inclusion and exclusion criteria. The screening visit will include a medical history, physical exam, height, weight, waist circumference measurements, vital signs, screening lab panel (fasting glucose, Chem profile, CBC, Lipid panel, TSH and urine microalbumin/creatinine).
All subjects will be counseled to take Aspirin 81-325 mg daily, to be physically active and smoking cessation will be recommended to active smokers. Subjects will continue to take all other concomitant medications at the same time taken prior to the start of this study.

A participant who is found to be ineligible during screening may be re-screened once within 3 months. If ineligible on the second screening for the same reason, the participant is permanently ineligible.

If a health issue is identified on the initial screen that leads to altered drug therapy, the participant may be re-screened, after establishing a new stable baseline.

**Baseline and End of Treatment (or Early Termination) Evaluation:**

Subjects will present to the General Clinical Research facility at the Beth Israel Deaconess Medical Center after a 12-hour fast, or the Clinical Research Center of the Joslin Diabetes Center for the salsalate stratum. Blood pressure will be assessed using a Dynamapp automated cuff with the subject sitting for 10 minutes. History, physical examination including, vital signs, height, weight, waist circumference measurements, electrocardiogram, fasting glucose, lipids (total cholesterol, triglycerides, HDL, LDL and free fatty acids), insulin, c-peptide, vascular and inflammatory markers hsCRP, IL6, TNF-α, PAI-1, adiponectin, serum amyloid A, the adhesion molecules ICAM and VCAM and serum nitrotyrosine as an assessment of oxidative stress. Blood will be stored for later analysis of inflammatory profiles, DNA and mononuclear lymphocyte RNA as well as immortalized lymphocytes among consenting participants. Subjects will then undergo Multi-detector computed tomographic angiography (MDCTA) to assess coronary plaque as fully described in the Imaging Core. Abdominal and liver adipose deposition will be measured by CT during MDCTA. Oral hydration after contrast agent will be initiated with one liter of water. Subjects who are unable to consume this volume orally may receive intravenous hydration. After baseline evaluation, study drugs will be dispensed. End of treatment assessment will be performed similarly at month 30. Two weeks before end of treatment visit, subjects will have blood drawn to check BUN, Cr and eCrCLCG. If eCrCLCG is <55 ml/min or serum Cr > 1.3 mg/dL, subjects will drink additional water for 3 days and have blood work repeated. If eCrCLCG continues to be <55 ml/min or serum Cr > 1.3 mg/dL, we will use the Beth Israel Deaconess Hospital clinical radiology protocol for hydration: Cr level 1.3 to 1.6 mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of Optiray 320 or 350, and for Cr level of 1.7 to 1.9 mg/dL: oral hydration pre and post administration of 100cc of Visipaque. At the end of the visit at month 30, subjects will be dismissed from the study and returned to the care of their primary and cardiac care physicians.

All attempts will be made to schedule an early termination visit for all subjects with non-urgent need to drop out of the trial (such as relocating out of the geographical area). Early termination visits will occur in an identical manner to the end of treatment evaluation.

**General Safety:** Subjects with a suspect critical lesion (significant obstructive disease of ≥ 70% in left main coronary artery, ostial LAD or three-vessel disease) on MDCTA will have these results immediately transmitted to their cardiologist and be referred for ETT and/or catheterization, if indicated, via referring cardiologist. Those with significant obstructive disease in left main coronary artery, ostial LAD or three-vessel disease will be excluded. They can then be reevaluated for inclusion/exclusion criteria after appropriate intervention if within 3 months of MDCTA.

**Lovaza Dosage and Administration:** Subjects will receive Lovaza 4 capsules a day. Subjects will continue to take any prescribed medication at the same time it was taken before the start of this study. Changes in dosing of all other medication will be avoided as possible according to best clinical practice guidelines throughout the trial.
Treatment Period (Month 0 to Month 30):

Subjects will be seen by their study physician at 6 month intervals. Coronary heart disease endpoints (myocardial infarction, angioplasty, bypass surgery, death from CVD, hospitalized angina or congestive heart failure) will be determined at 3, 6, 9, 12, 18, 24 and 30 months. We will have an endpoint manual with algorithms for each event. Participants would also be monitored by telephone by the study coordinator twice-monthly the first month following randomization then monthly thereafter, to facilitate drug dispensing and compliance and to schedule safety blood monitoring through Quest at 12 months. Subjects will be asked about concomitant medications and any adverse events. The study coordinator will ship out an additional supply of study drugs and then get the subjects to return all unused pills by mailer so that pills may be counted to assess compliance. (See Table 12).

Patient Follow-up  Patients will have fasting safety bloods drawn at baseline (visit 2), 12 months, 24 months and end of study 30 months for the lifestyle trial and at baseline (visit 2), 6 weeks (visit 3), 3 months (visit 4), 6 months (visit 5), 9 months (visit 6), 12 months (visit 7), 18 months (visit 8), 24 months (visit 9), and 30 months for the salsalate trial. Interim laboratory assessment can be performed at the local Quest laboratory, cardiology site office, BIDMC or JDC (fasting glucose, comprehensive metabolic panel, liver enzymes and CBC). These blood draws will be scheduled by the study coordinator and each visit will be preceded and followed with a telephone call from the study coordinator/study nurse (see table 13 for lab schedule). At the time of the telephone call after the laboratory tests, the patient will be queried about status, change in medication, and any adverse events, physician visits, hospitalizations, or other events. Subjects admitted to the hospital will be asked to sign a release form, and copies of records and discharge summaries will be obtained to assess for adverse reactions and cardiac endpoints such as hospitalized angina, heart failure, myocardial infarction, angioplasty, or coronary artery bypass grafting. Pills will be shipped out after randomization and every 4 months thereafter directly to the patient, with the instruction to see the cardiologist, and an appointment will be scheduled. Patients will be seen at their Cardiology office/BIDMC/JDC at 6 months, 12 months, 18 months, and 24 months-and vital signs, questionnaire administration, and pill count will be carried out at that time. All patients will also have a final closeout visit at BIDMC/JDC with fasting blood work, history (questionnaires) and physical examination, and final MDCTA at that time (visit 10-final close out visit). At each visit, information will be obtained on adverse reactions, hospitalizations and endpoints.

Subjects will speak by phone at scheduled intervals, 6 weeks, 3, 6, 9, 12, 18, and 24 months for potential adverse events. 30-month endpoint assessment will be performed by study investigators at the closing visit. If the subject has any type of adverse event, they need to call

Table 13: Laboratory Schedule for Lifestyle Arm

<table>
<thead>
<tr>
<th>Visit Number</th>
<th>Screening</th>
<th>Baseline</th>
<th>Active treatment Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit Number</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Month</td>
<td>-4 months to -1 week</td>
<td>0</td>
<td>Week 6</td>
</tr>
<tr>
<td>HbA1c</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Lipid Profile (Chol. Trig, LDL-C, HDL-C)</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Insulin</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-peptide</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>TSH</td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Chemistry profile</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CBC with automated differential</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Urine microalbumin/creatinine</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

(Chem profile:  NA, K, CL, Bicarb, Bun, Cr, ALT, AST, LDH, Alk phos, Total Bili, Ca, Phos, Uric Acid, Total Pro, Alb)
their primary doctor immediately and the study coordinator subsequently (see algorithm). Subjects will present to the office of their clinical cardiologist for visits every 6 months including history, physical examination, vital signs including weight and blood pressure. DSMP is described below.

Finally, subjects will be followed by telephone at 6 month intervals for 2.5 years after the 30-month visit (for a total of 5 years follow-up), to continue to assess the effect of interventions on cardiovascular outcomes.

2.3 Overview of IKKβ inhibition using salsalate:

The novel link between activation of the inflammatory IKK/NF-κB pathway and insulin resistance is established in animal models of obesity and diabetes. We propose to inhibit the IKK/NF-κB pathway using the salicylate, salsalate (which has an established long term safety profile for human use), to promote vascular remodeling assessed as reduction in soft plaque measured with MDCTA. We predict that improvement in insulin sensitivity, and reduction in CRP and triglycerides induced by inhibition of the IKK/NF-κB pathway will promote vascular remodeling.

The Joslin Diabetes Center will be the coordinating center for the salsalate/placebo intervention. The conduct of this arm of the study will be identical in nature to the lifestyle arm. The proposed study will follow a randomized, double-masked, placebo-controlled, parallel-group trial design. Inhibition of IKKβ/NF-κB in overweight/obese patients with known coronary artery disease will be achieved using the salicylate, salsalate (Disalcid) dosed at 3.5 g daily, divided, for a 30-month treatment period. Approximately 139 patients will be randomly assigned to salsalate and 139 patients to salsalate placebo. There will be a 4-month screening period, pre-treatment baseline evaluation, and a 30-month double-blind treatment period followed by an end of treatment evaluation. The baseline and end of treatment evaluations will consist of fasting physical and laboratory measures and assessment of calcified and soft plaque in the coronary arteries by Multi-detector computed tomographic angiography, as described fully below.

2.3.1 Study Design:

This is a 31-month, randomized, double-masked, placebo-controlled, parallel-group study with a 4 month screening period, and a 30-month double-masked treatment period and 4-week post treatment follow-up. There will be a total of 10 visits. The baseline, 12 month, and final evaluation will be performed at the General Clinical Research Center (GCRC) of the Beth Israel Deaconess Medical Center (BIDMC) /Joslin Diabetes Center (JDC). Interim visits will occur in the office of the patient’s cardiologist care provider or JDC. The trial schedule is identical to that above.

2.3.2 Subject Identification:

Patients will be identified similarly as described in the lifestyle trial through cardiology practice sites participating in this research project, or from the clinical cardiologists at the BIDMC, or the JDC clinic or by census mailing. The consortium of sites will be selected among those located in the Northeast so that subject can commute to the BIDMC/JDC for baseline and end of treatment evaluation with minimal inconvenience. Study staff will also perform protocol and site review with the clinical staff, coordinate sites with local or centralized institutional review board, manage site budgets, provide oversight for investigator compliance and attaining patient enrollment goals review, and collection of regulatory documents.

Again, cardiology sites will require centralized IRB New England Institutional Review Board (NEIRB), Wellesley, MA. BIDMC approved consent documents will be provided to the
centralized IRB to maintain uniformity. However, subjects will be re-consented by a study team member at the time of the first visit to the BIDMC.

2.3.3 Visit Schedule

Screening Visit: The Screening visit will occur between -4 months and –1 week at BIDMC/JDC/cardiology sites. After explaining the nature of this study in detail, written informed consent will be obtained before any study procedure being performed. Subjects will be screened for inclusion and exclusion criteria. The screening visit will include a medical history, physical exam, height, weight, waist circumference measurements, vital signs, screening lab panel (fasting glucose, Chem profile, CBC, Lipid panel, TSH and urine microalbumin/creatinine) and rectal exam for stool guaiac.

All subjects will be counseled to take Aspirin 81-325 mg daily, to be physically active and smoking cessation will be recommended to active smokers. Subjects will continue to take all other concomitant medications at the same time taken prior to the start of this study.

A participant who is found to be ineligible during screening may be re-screened once within 3 months. If ineligible on the second screening for the same reason, the participant is permanently ineligible.

If a health issue is identified on the initial screen that leads to altered drug therapy, the participant may be re-screened, after establishing a new stable baseline.

Baseline and End of Treatment (or Early Termination) Evaluation: Subjects will present to the Clinical Research Center at the Joslin Diabetes Center after a 12-hour fast. Blood pressure will be assessed using a Dynamapp automated cuff with the subject supine for 10 minutes. History, physical examination including, vital signs, height, weight, waist circumference measurements, electrocardiogram, fasting glucose, lipids (total cholesterol, triglycerides, HDL, LDL and free fatty acids), insulin, c-peptide, vascular and inflammatory markers hsCRP, IL6, TNF-α, PAI-1, adiponectin, serum amyloid A, the adhesion molecules ICAM and VCAM and serum nitrotyrosine as an assessment of oxidative stress. Blood will be stored for later analysis of inflammatory profiles, DNA and mononuclear lymphocyte RNA as well as immortalized lymphocytes among consenting participants. Subjects will then undergo Multi-detector computed tomographic angiography (MDCTA) to assess coronary plaque as fully described in the Imaging Core. Abdominal and liver adipose deposition will be measured by CT during MDCTA. Oral hydration after contrast agent will be initiated with one liter of water. Subjects who are unable to consume this volume orally may receive intravenous hydration. After baseline evaluation, study drugs will be dispensed. End of treatment assessment will be performed similarly at month 30. Two weeks before end of treatment visit, subjects will have blood drawn to check BUN, Cr and eCrCLCG. If eCrCLCG is <55 ml/min or serum Cr >1.3 mg/dL, subjects will drink additional water for 3 days and have blood work repeated. If eCrCLCG continues to be <55 ml/min or serum Cr >1.3 mg/dL, we will use the clinical radiology protocol for hydration: Cr level 1.3 to 1.6mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of Optiray 320 or 350 and for Cr level of 1.7 to 1.9 mg/dL: oral hydration pre and post administration of 100cc of Visipaque. At the end of the visit at month 30, subjects will be dismissed from the study and returned to the care of their primary and cardiac care physicians.

All attempts will be made to schedule an early termination visit for all subjects with non-urgent need to drop out of the trial (such as: relocating out of the geographical area). Early termination visits will occur in an identical manner to the end of treatment evaluation. MDCTA will be repeated for participants who have completed one year or more of the trial following randomization.
General Safety: Subjects with a suspect critical lesion (significant obstructive disease of \( \geq 70\% \) in left main coronary artery, ostial LAD or three-vessel disease) on MDCTA will have these results immediately transmitted to their cardiologist and be referred for ETT and/or catheterization, if indicated, via referring cardiologist. Those with significant obstructive disease in left main coronary artery, ostial LAD or three-vessel disease will be excluded. They can then be reevaluated for inclusion/exclusion criteria after appropriate intervention if within 3 months of MDCTA.

All attempts will be made to schedule an early termination visit for all subjects with non-urgent need to drop out of the trial (such as: relocating out of the geographical area. Early termination visits will occur in an identical manner to the end of treatment evaluation.

After baseline evaluation, study drug/placebo will be dispensed. End of treatment assessment will be performed similarly at month 30. At the end of the visit at month 30, subjects will be dismissed from

Double-Masked Treatment Period (Month 0 to Month 30): Subjects will be seen by their study physician at 6 month intervals. Coronary heart disease endpoints (myocardial infarction, angioplasty, bypass surgery, death from CVD, hospitalized angina or congestive heart failure) will be determined at 3, 6, 9, 12, 18, 24, and 30 months. Participants would also be monitored by telephone by the study coordinator twice the first month following randomization then monthly thereafter, both to facilitate drug dispensing and compliance, and to schedule of laboratory assessments at 6 weeks, 3, 6, 9, 12, 18, 24, and 30 months. the study and returned to the care

Table 14: Laboratory Schedule for Salsalate and Placebo Arms

<table>
<thead>
<tr>
<th>Visit Number</th>
<th>Screening</th>
<th>Baseline</th>
<th>Active Treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>-4 months to -1 week</td>
<td>0</td>
<td>Week 6 3 6 9 12 18 24 30</td>
</tr>
<tr>
<td>Salicylates</td>
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<td></td>
<td>( \times ) x x x x x x x x x</td>
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<tr>
<td>Hba1c</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Lipid Profile (Chol. Trig, LDL-C, HDL-C)</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>x</td>
<td>( \times ) ( \times )</td>
</tr>
<tr>
<td>C-peptide</td>
<td></td>
<td>x</td>
<td>( \times ) ( \times )</td>
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<tr>
<td>TSH</td>
<td></td>
<td>x</td>
<td>( \times ) ( \times )</td>
</tr>
<tr>
<td>Chemistry profile</td>
<td>x</td>
<td>x</td>
<td>( \times ) ( \times )</td>
</tr>
<tr>
<td>CBC with automated differential</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine microalbumin/creatinine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Chem profile: NA, K, CL, Bicarb, Bun, Cr, ALT, AST, LDH, Alk phos, Total Bill, Ca, Phos, Uric Acid, Total Pro, Alb)

* Laboratory evaluation 2-4 weeks after end of 30-months treatment will be performed in subjects who fulfill either of the following conditions:

a) At the final dosing visit a decline in eGFR by 40 ml/min from baseline or a serum creatinine > 1.5 mg/dl in women and 1.6 in men.

b) At the final dosing visit a systolic blood pressure >160, a diastolic blood pressure >95, or a change in systolic or diastolic blood pressure of >10 mm Hg from baseline.

\(^{1}\)HbA1c at these time points will only be performed for patients with diabetes mellitus.

of their primary and cardiac care physicians. Subjects will be asked about concomitant medications and any adverse events. The study coordinator/pharmacy will ship out an additional supply of study drugs and then get the subjects to return all unused pills by mailer so that pills may be counted to assess compliance. Subjects will have safety blood monitoring through Quest locally at 6 weeks, 3 months, 6 months, 9 months, 12 months, 18 months, and 24
months, with the 30 months endpoint evaluation during the final visit. (See Table 14 for laboratory schedule for the salsalate and placebo arms of the trial).

Post treatment follow-up: All participants receive post treatment follow-up either by phone or by an in-clinic visit, which is scheduled 4 weeks after the final visit to review their interim health status. Follow-up is by phone unless either of the following conditions is met:

At the final dosing visit a decline in eCrCLCG by 40 ml/min from baseline (i.e. greater than the anticipated maximum decline of 10 ml/min per year) or a serum creatinine > 1.5 mg/dl in women and 1.6 in men.

At the final dosing visit a systolic blood pressure >160, a diastolic blood pressure >95, or a change in systolic or diastolic blood pressure of >10 mm Hg from baseline.

Participants who meet either of the above criteria are scheduled for an in-clinic visit to obtain an interim medical history, vital signs, and chem 7 (electrolytes, bun/cr and glucose).

2.3.4 Study Drug Dosage and Administration:

Subjects will be randomly assigned to receive salsalate 3.5 g/day administered as 500 mg tablets, divided into 2 doses per oral daily, or placebo, for 30 months. Placebo tablets will look identical to the active tablets. Placebo and active tablets will be made by Caraco Pharmaceutical Laboratories, Ltd, Detroit, MI, USA.

Subjects will continue to take any prescribed medication at the same time it was taken prior to the start of this study. Changes in dosing of all other medication will be avoided as possible according to best clinical practice guidelines throughout the trial. Subjects will speak by phone at scheduled intervals, 6 weeks, 3, 6, 9, 12, 18, and 24 months for potential adverse events. 30-month endpoint assessment will be performed by Study Investigators at the closing visit. Subjects with adverse events identified on the interim follow-up will be scheduled to see a study investigator or their care provider. Laboratory assessments for safety variables will be performed at 6 weeks, 3, 6, 9, 12, 18, 24, and 30 months. Subjects will present to the office of their clinical cardiologist for visits every 6 months visits including history, physical examination, vital signs including weight and blood pressure. While salsalate is widely prescribed for pain management and there is no reported contraindication to prescription of salsalate and medication used to treat atherosclerosis, renal function and anion gap will be monitored closely at scheduled intervals due to the effect the agent may have on creatinine and acidosis. Elevation in creatinine, or decline in eCrCLCG < 50 ml/min, or development of anion gap acidosis will lead to a decrease in salsalate dose or early termination from the study.

Finally, subjects will be followed by telephone at 6 month intervals for 2.5 years after the 30-month visit (for a total of 5 years follow-up), to continue to assess the effect of interventions on cardiovascular outcomes.

All study subjects will be recommended to continue follow-up with their clinical cardiologist every 6 months. We will recommend all providers participating in the study follow standard of care treatments for participants with heart disease, in accordance with the guidelines of the American College of Cardiology, American Heart Association and American Diabetes Association guidelines for treatment of patients with coronary heart disease (encouraged to follow good medical practice guidelines).

The data safety monitoring plan (DSMP) is described in detail in the appropriate section of the CCI application. DSMB is has been established as recommended by the NHLBI guidelines.
2.3.5 Outcome Assessments:

Compelling evidence from several major clinical epidemiology studies suggests that increased inflammation and insulin resistance precede and predict cardiovascular morbidity and mortality. Reductions in the inflammatory marker CRP, one of many secondary endpoints in this study, are independently associated with reduced rate of progression of plaque assessed by intravascular ultrasound [293] and treatment of insulin resistance per se with either the biguanide metformin or thiazolidinedione class agents leads to lower insulin, inflammatory and fibrinolytic markers and have beneficial effects on vascular function in vivo. The proposed study will evaluate the role of inhibition of the IKKβ/NF-κB pathway to reduce serum levels of CRP and promote vascular remodeling and regression of soft plaque in overweight/obese patients with coronary artery disease. Confirmation that salsalate, an inexpensive, generally well tolerated agent with a long standing safety profile in human use, is effective would have significant health and economic implications in the treatment of this disorder.

Overweight/obese subjects with coronary artery disease will be studied before and after treatment with salsalate/placebo. Subjects will randomly be assigned to receive salsalate 3.5 g/daily, for 30 months.

**Primary Outcome:** The primary outcome will measure change in soft plaque measured by Computed Tomography (CT) angiography. Sample size determination is based on definition of the primary outcome and method of analysis. We will use methods based change from baseline to determine sample size for the trial with two-sample t-test using each patient’s change from baseline score to compare differences in treatment groups between baseline and fixed (final) time points. Details of secondary endpoints and statistical analysis are provided below (Chapter 3).

2.3.6 Early Termination

Study subjects will be encouraged to complete the study. However, all efforts will be made to collect all end of study measures for subjects seeking to withdraw participation prior to 30 months. All subjects who have had at least 12 months of participation following randomization, will be requested to undergo follow-up MDCT.

2.3.7 Evaluation for Potential Toxicity & Side Effects Associated with Salsalate

**Safety measures:** As discussed above, subjects will have a complete medical history and physical examination at the screening visit, baseline (prior to dispensing the drug/placebo) and end of treatment, and interim every 6 months throughout the study. Vital signs will be assessed at each visit. Laboratory safety measures (chemistry, including Na, K, CL, Bicarb, Bun, Cr, ALT, AST, LDH, Alk phos, Total Bili, Ca, Phos, Uric Acid, Total Pro and Alb) will be measured at screening, prior to randomization and at 6 weeks, 3, 6, 9, 12, 18, 24, and 30 months during drug/placebo administration for the salsalate and placebo arms of the study and at 12, 24 and 30 months for the lifestyle trial of the study. Urinary albumin will be assessed at 6, 12, 18, 24 and 30 months, HbA1c will be followed at the same intervals only in patients with established diabetes. Complete blood cell counts will be performed at baseline, 12, 24 and 30 months. Adverse events will be assessed at each visit and by phone at 6 weeks, 3, 6, 9, 12, 18, 24 and 30 months. Subjects who report adverse events during interim communications will be scheduled to meet with a study investigator.

**Pharmacokinetics:** Salicylate metabolism is complex. Preliminary studies suggest that the salicylate level achieved at 3.0 g/daily may be lower in patients with diabetes than in the general population. This could be due to enhanced renal clearance with hyperfiltration or glycosuria,
renal tubular acidosis, or partitioning into adipose tissues. Salicylate levels will be measured 6 weeks, 3, 6, 9, 12, 18, 24, and 30 months and time from last dose recorded. Data safety monitor will review doses and recommend decrease for levels greater than 30 mg/dl, to maintain masking, for every 2 subjects reduced in active treatment, a subject in the placebo group will also have a reduction in dose recommended by the safety monitor.

Risk of Tinnitus: The most common adverse event associated with salsalate use is tinnitus (Caraco Pharmaceuticals, Package Insert). In a clinical efficacy study about 10% (13/131) of patients receiving salsalate discontinued therapy due to tinnitus [286]. After 12 weeks of therapy the respective median and mean salsalate dose was 3 and 3.3 grams daily (range 2-4.5 grams). Cessation of treatment resulted in disappearance of tinnitus.

Risk of Hypoglycemia: Preliminary data demonstrates salsalate can improve insulin sensitivity. In contrast to insulin secretagogues, insulin-sensitizing agents are less likely to induce hypoglycemia. Insulin sensitizers including metformin andTZDs have been safely administered to persons with modest elevations of blood sugar, for example subjects with impaired glucose tolerance but no overt diabetes. Hypoglycemia was not seen in the preliminary studies with salsalate in subjects with T2D or impaired glucose tolerance, but has been reported to occur with salicylate overdose in pediatric populations. This may be due to liver dysfunction seen in Reyes syndrome. Persons under 18 years of age will not be eligible to participate in this study. Weight loss and increased exercise can also improve insulin sensitivity and may predispose subjects on medications such as sulfonylurea or insulin to hypoglycemia. Nevertheless, subjects will be educated in the signs and symptoms of hypoglycemia at the time of the first visit and instructed to notify the investigator or care provider if symptoms occur.

Risk of Gastrointestinal Distress or Bleeding. In contrast to aspirin, salsalate is not soluble in the acid environment of the stomach, but is hydrolyzed into two molecules of salicylic acid and absorbed in the alkaline environment of the small intestine. This reduces the incidence of gastrointestinal intolerance. Although GI side effects have been reported with salsalate, they tend to occur in people with pre-existing GI disease [294]. Salsalate causes no greater intestinal occult blood than placebo [212, 295] and does not inhibit platelet aggregation [296]. Salsalate neither prolongs prothrombin nor bleeding times [211]. Stool guaiac will be performed at baseline and at 6-month intervals throughout the study. Complete Blood Counts will be performed at baseline, 12 and 30 months.

Risk of Bronchospasm. Salsalate is less likely than aspirin to induce bronchospasm. 3M, the previous manufacturer of salsalate and Caraco, the current manufacturer, report infrequent occurrence of bronchospasm with salsalate (personal communications). However, persons with aspirin intolerance will be excluded from study participation.

Altered renal, liver, or hematological function. Salsalate has been extensively prescribed without untoward effects. There is no known contraindication to use in patients with known cardiovascular disease, diabetes, or features of the metabolic syndrome. Hematology and chemistry profiles will be assessed at baseline and throughout the study as described. Although creatinine remained within normal limits, we have seen small but significant increases during the 2-week ASA and salsalate trials. Hence, serum creatinine will be closely monitored at interim visits and a decrease in eCrClCG to <50 ml/min will warrant dose reduction by 500 mg increment.

Estimation of Glomerular Filtration Rate: It has been recognized that renal dysfunction begins before the serum creatinine become abnormal. Glomerular Filtration Rate (GFR) is difficult to measure as most metabolic products are both filtered and reabsorbed through the kidney. Clinical estimates of renal function for decades have been principally based on the 24 hour urine measure of creatinine clearance. Recently, the formula-derived estimates of
Cockcroft-Gault (CG) and Modification of Diet in Renal Disease (MDRD) have been increasingly employed. It is important to note that the Cockcroft-Gault formula was derived to estimate creatinine clearance [297] while the MDRD estimates measured GFR [298].

The performance of the CG and MDRD equations have been compared with measured $^{125}$I-iothalamate GFR (iGFR) in a large study of patients with chronic kidney disease (CKD) and in potential kidney donors [292]. In patients with established CKD (GFR <60 ml/min per 1.73 m2), the MDRD equation has been shown to perform better than the CG formula. However, the group without established renal dysfunction, the MDRD equation significantly underestimated the measured GFR when compared with the CG formula.

Despite the increasing use of the conservative MDRD eGFR estimate, we propose to use the CG equation for the following reasons: (1) First, we do not intend to enroll participants with established renal disease. (2) Second, NSAIDs as a class have been widely prescribed to patients based on normal creatinine, and not clinically restricted based on eGFR. This clinical use persists despite the association of NSAID as a pharmaceutical class being associated with impaired renal function. (3) Third, the NSAID effect on the kidney is attributed to inhibition of COX enzymes and thereby renal prostaglandins. Salsalate is distinct among the NSAID as it does not inhibit COX. Furthermore, salsalate has no suppressive effect on renal prostaglandin production, measured by plasma rennin activity, while aspirin and naproxen showed definite suppression (34% and 49%, respectively) [299]. Finally (4) recent large population based studies, including the Third National Health Nutrition and Examination Survey (NHANES III) and the Atherosclerosis Risk in Communities (ARIC) study, have demonstrated an association between the metabolic syndrome and Chronic Kidney Disease (CKD) [300, 301]. The prevalence of CDK increases stepwise with the number of metabolic syndrome traits, such that risk of CDK is highest for those with all 5 metabolic syndrome traits. If CDK represents a newly recognized component of the cluster of associated metabolic conditions it is conceivable that diet induced obesity causing subacute chronic inflammation could underlie CDK. If such, then treatment of inflammation may actually improve renal function.

We will carefully monitor renal function over the course of the trial. However, we do not want to compromise the integrity of the study based on overestimation of renal dysfunction. This would lead to exclusion of persons with normal creatinine who may benefit from participation and in whom, over the many years of clinical use of salsalate, have not demonstrated significant additional renal compromise.

Cockcroft-Gault Equation: $\text{eCrCL}_{\text{CG}}$ (ml/min) = $\frac{[(140 - \text{age}) \times \text{weight (kg)}][\text{SCr (mg/dl)} \times 72]}{[0.85 \text{ if female}]}$. 

\[ \frac{140 - \text{age} \times \text{weight (kg)}}{[\text{SCr (mg/dl)} \times 72}] \times [0.85 \text{ if female}] \]
Chapter 3: Statistical Considerations

3.1 Sample Size Estimates

Sample Size Estimates are again provided in the Study Overview Figures (figures 26). For the lifestyle trial we expect to screen approximately 340 subjects and for the salsalate trial we expect to screen approximately 389 subjects identified at cardiology sites and census mailing (note that patients seen in the cardiology clinics at the BIDMC will also be able to be screened and enrolled. If screen failures exceed the predicted 10-20% rate we will notify the Committee on Clinical Investigation. We hope to perform baseline evaluation on 313 subjects in the lifestyle trial and 313 subjects in the salsalate trial. We anticipate that 10%-11% of MDCTA scans will not be of adequate quality to permit longitudinal evaluation. Thus, 278 subjects will be enrolled in the lifestyle trial and 278 subjects in salsalate trial.

3.2 Randomization, Stratification, and Masking:

* Some sites participating in this trial will refer study subjects only to the salsalate portion of the study. If subjects from a site participating only in the salsalate arm are found to be ineligible for the salsalate portion of the study they will be only be asked for permission to provide their contact information to the Lifestyle arm team only with approval of their referring care provider.
The study will use permuted block randomization, based on the presence of diabetes. As this is a significant cardiac risk factor that is synergistic to cardiac event risk with hypertension and hypercholesterolemia, disproportionate randomization could skew results. We will adjust any imbalances that may occur across groups on other characteristics using adjustments in the analysis. With permuted blocks, excessive stratification increases the likelihood of unfilled blocks thus increasing the chance of imbalances.

Masking can be implemented at many levels. In the lifestyle modification protocol, blinding is impossible since study subjects undergoing the intensive lifestyle recommendations or the usual care will know. Randomization will be performed by the study statistical collaborators, Dr. Thomas Hauser or Griffin Weber, M.D.,PhD.

3.3 Primary Outcome:

The primary outcome will measure change in soft plaque measured by Computed Tomography (CT) angiography between baseline and 30 months on a per plaque basis. This is a change from our original primary endpoint which was change in plaque volume per patient. The reason for the change is that data since our grant submission has shown that plaque progression and regression is greatly influenced by shear stress forces which vary from one plaque to another depending on the location of the plaque in the artery [302, 303].

3.4 Sample size determination

The power of this study to detect a change in coronary artery plaque volume is estimated based on the preliminary data for the lifestyle intervention. There is no direct preliminary data available regarding the effects of salsalate therapy on coronary atherosclerosis. The lifestyle preliminary data showed a 9.3% decrease in LDL cholesterol [182]. As the majority of the patients in this trial will be on statin therapy, we expect a 30% smaller treatment effect, resulting in a reduction in LDL cholesterol of 6.5%. Coronary CTA data assessing reduction in coronary artery plaque volume with statin therapy has shown a 24 ±13% reduction in plaque volume with a 40.5% reduction in LDL cholesterol in 27 patients [304]. Assuming a linear relationship between LDL cholesterol reduction and reduction in coronary artery plaque volume [305, 306], this trial expects to observe a 3.9% reduction in plaque volume in the active treatment group. Assuming no change in plaque volume in the placebo group and an average of 1.7 measurable plaques per study subject, the number of subjects required to have a type II error of 0.2 (80% power) with a type I error of 0.05 (alpha 0.05) is 104 per treatment arm. This analysis assumes that plaque volume changes are independent within each study subject. Data from prior invasive angiographic trials suggests that this is not the case, with an absolute correlation of up to 0.24[307]. Assuming that the intra-subject correlation is 0.24, the appropriate variance inflation factor is 1.06 to account for this lack of statistical independence [308]. Thus, a total of 111 subjects is required for each treatment arm. With an initial randomization of 139 subjects to each arm and a 20% dropout rate, resulting in a total of 111 subjects with assessment of the primary endpoint, this study will have a power of 0.80 to detect a 3.9% difference in rate of plaque progression between active and placebo/control. To account for our 18% screen and baseline failure rate, we will enroll 170 per arm per trial (680 total for both trials).

3.5 Secondary Analysis and Outcomes:

Secondary Analysis:

Secondary analysis in the salsalate stratum will include two per protocol analyses, one with those on full dose and one including maximum tolerated dose to better understand both efficacy and safety finding related to drug exposure.
Secondary Outcomes

Important secondary outcomes include:

Coronary artery plaque assessments:

1) Baseline and change over 30 months in percent atheroma volume (PAV) calculated as the proportion of the entire vessel wall occupied by atherosclerotic plaque; and total atheroma volume, normalized to segment length.

2) Baseline and change over 30 months in maximum percent diameter stenosis and minimal luminal diameter.

3) Comparisons of number and subjects with categorical variables of maximal stenosis >50% and number with 3-vessel disease >20%.

4) Baseline and change over 30 months in the remodeling index will be calculated by the ratio of plaque volume at the most diseased site compared to the least diseased site within the proximal 10 mm of vessel at baseline and end of study.

Metabolic measures:

19) Improvement in parameters of the metabolic syndrome assessed by measures by waist circumference, systolic and diastolic blood pressure, lipid profiles (total cholesterol, triglycerides, HDL and LDL), and abdominal adiposity quantitated by computerized tomography.

20) Reduction of mediators of inflammation in the circulation including CRP, PAI-1, serum amyloid A, MMP-9 and fibrinogen, pro-inflammatory cytokines including IL-6, TNF-a and IL-1b, the adhesion molecules VCAM-1 and ICAM-1, increase in adiponectin and reduction in serum nitrotyrosine as a marker of oxidative stress.

21) Reduction of insulin resistance assessed by fasting insulin or C-peptide and homeostasis model assessment of insulin resistance (HOMA-IR).

22) Reduction of inflammation in the liver associated with nonalcoholic steatohepatitis (NASH), a newly recognized component of the metabolic syndrome, and reduction of fatty liver quantitated by computerized tomography and levels of AST and ALT as markers of liver inflammation related to NASH.

23) Comparison of rates of addition of anti-hypertensive, diabetic, or lipid lowering medication.

24) Comparison of numbers of persons with metabolic syndrome who progress to diabetes between groups.

25) Comparison of numbers of persons who regress from ATPIII metabolic syndrome criteria. (for those with metabolic syndrome).

26) Investigation of the relationship between vitamin D status and coronary calcification, as well as with insulin resistance (HOMA-IR), beta-cell function (HOMA-%beta), and serum levels of inflammatory cytokines and adhesion molecules, known to be related to CVD risk.

27) Determination of whether baseline vitamin D levels predict clinical response to salsalate, and whether hypovitaminosis D is associated with plaque progression.

3.6 Statistical Analysis

This trial assesses the change in coronary plaque volume as measured by MDCTA. The primary analytic approach will be to use student’s t-test to compare each of the active treatment groups to their respective standard of care /placebo group. Statistical significance will be
assessed using a p-value of 0.05. The primary analysis will be performed on an intent-to-treat basis amongst those subjects who have had the primary endpoint evaluated (change in coronary plaque volume during the study, requiring 2 measurements). For the primary endpoint, there is no easy way to address missing data. It is not possible to carry forward values if there is only a baseline measurement. Therefore, patients who do not return for a second MDCTA will be treated as missing data. Because missing data may represent informative censoring, we will perform a formal survival analysis of both the intervention and placebo groups to determine that there has been no biased loss to follow-up. Additionally, we will perform a sensitivity analysis imputing conservative values for missing data (e.g. the mean change observed in the control group) and optimistic values (e.g. mean change observed in the active treatment group for missing data in the active treatment group) to further assess the magnitude of any biased losses. We will then impute a follow-up value based on the mean CRP change observed in the control group (a relatively conservative assumption). For certain secondary outcomes, the last observation will be carried forward.

Given the potential for noncompliance with the lifestyle intervention, we will also perform a pre-specified secondary analysis on a per protocol basis. If the primary intent-to-treat analysis is negative (non-significant), the per-protocol analysis will aid in determining if the results are significantly affected by noncompliance.

Analyses of the secondary outcomes are exploratory and will be performed using multiple methods, including ANOVA, linear regression, logistic regression, longitudinal regression, and time to event analyses. We will allow assessment of secondary endpoint laboratory markers as early as 6 months into the study, as these have a much more rapid response to therapy.

We will assess the effects of missing data in the final dataset using a sensitivity analysis, imputing missing values using various optimistic and pessimistic assumptions (no change, mean/median change in the active treatment arm, mean/median change in the standard care arm, etc.) to assess the magnitude of any biased losses. Data from early assessment of the primary outcome in those participants who drop out of the trial will be used to determine the validity of these assumptions. We will also perform multiple imputation analysis using standard algorithms. Because missing data may represent informative censoring, we will also perform a formal survival analysis of both the intervention and placebo groups to determine if there has been any biased loss to follow-up.

Biostatistical support will be provided by: Dr. Shiva Gautam and Dr. Thomas Hauser and data management support with Dr. Steve Berry and Griffin Weber M.D., PhD.

1. Hans Hoffman, Katie Frieler, Peter Schlattmann, Bern Hamm, Marc Dewey. Influence of statin treatment on coronary atherosclerosis visualized using multidetector computed tomography; European Society of Radiology July 18, 2010 online.
CHAPTER 4: STUDY SUBJECTS

4.1 Inclusion Criteria

For the salsalate stratum: Eligibility criteria will be based upon the presence of established coronary artery disease including previous myocardial infarction (≥6 months ago), previous coronary bypass surgery (> 12 months ago), or angioplasty, stable angina, or evidence of coronary artery disease on prior imaging studies including, plaque in at least one coronary artery, or abnormal exercise tolerance test, or an area of reversible ischemia on nuclear imaging study or pharmacologic stress, with subsequent revascularization or determined not to require intervention by care providing cardiologist, or abnormal exercise treadmill stress test with or without nuclear imaging or echocardiography with the following exclusions:

Exclusions based on nuclear imaging:
1. Transient cavity dilation
2. More than one vascular territory involved with reversible defect (multiple defects)
3. Reversible defects involving the anterior wall, septum or apex (LAD territory)

Exclusions based on echocardiography imaging:
1. More than one vascular territory involved with inducible wall motion abnormalities (multiple defects)
2. Inducible wall motion abnormalities involving the anterior wall, septum or apex (LAD territory)

Subjects should be at least 6 months after a myocardial infarction and/or revascularization procedure to be eligible.

For the Lifestyle stratum Eligibility will be based upon the presence of established coronary artery disease including previous myocardial infarction (≥6 months ago), previous coronary bypass surgery (> 12 months ago), stable angina, significant non-calcified plaque (as determined by Dr. Clouse) in at least one coronary artery or abnormal exercise tolerance test or an area of reversible ischemia on nuclear imaging study or pharmacologic stress, with subsequent revascularization, or angioplasty, or abnormal exercise treadmill stress test with or without nuclear imaging or echocardiography with the same exclusion listed immediately above.

All subjects must also have either a BMI of ≥ 27 kg/m2 (26.6 is rounded to 27) as a surrogate of the metabolic syndrome or a BMI of 25 to 26.5 with an elevated C-reactive protein or with increased waist. Assessment of increased waist will be determined based on the criteria described in “Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and blood Institute; American Heart Association,; World Heart Federation; International Atherosclerosis Society; and international association for the Study of Obesity” guidelines, or for lifestyle with 2 additional components of metabolic syndrome. (to prevent under-representation of minority persons in our investigations, we will use a BMI ≥24.5 for subjects from Asian origin [291])

In addition, subjects must be: a) aged 21- 75 years inclusive, b) have BMI ≤ 35 kg/m² if females and ≤ 40 kg/m² if males, c) on a stable dose of an HMG CoA reductase inhibitor (statin) for 1 month at screening or unable to tolerate a statin, d) have normal renal function, (note estimated creatinine clearance calculated using Cockcroft-Gault (CG) [292] equation ≥60 at screening [\[\text{eCrCLCG (ml/min)} = \frac{[(140 - \text{age}) \times \text{weight (kg)}]}{[\text{Scr(mg/dl)} \times 72]} \times [0.85 \text{ if female}]] \text{ serum creatinine of } \leq 1.3 \text{ if CrCl is } < 60 \text{ ml/min/1.73m}^2 \text{ and there is no history of renal problems, e)
have liver function (ALT, AST) < 3 times upper limits of normal), f) normal thyroid function (on stable dose replacement therapy is acceptable), g) if women are of child bearing potential they must have a pregnancy test prior to the CT angio and use contraception for the remainder of the study h) For those entering lifestyle an ETT performed within 12 months providing there has been no increase in their anginal pattern; if there has been a change in their anginal pattern over the 12 months prior to the study, study subjects will need a new ETT. and i) For the salsalate trial patients with T2D must have a fasting glucose of ≤ 200 mg/dl at screening and cannot be treated with thiazolidinedione class agents or insulin or Exendin-4 (Byetta) therapy.

Subjects must be willing to have two visits at the Beth Israel-Deaconess Medical Center with a baseline and a 30-month follow-up series of imaging studies including CT angiography of the coronary arteries and imaging of the aorta, abdominal adiposity and liver.

Subjects with Medicare health insurance will have equal opportunity to participate in this study. Considering their older age, Medicare subjects are more likely to be diagnosed with coronary heart disease and therefore the results of this study will be especially applicable to the Medicare population.

4.2 Exclusion Criteria

Exclusion criteria include:

1) Unstable angina (increase in frequency or severity of anginal episodes or development of chest pain at rest)

2) Significant obstructive disease (>70%) in left main coronary artery, ostial LAD or three-vessel disease by MDCTA

3) Significant heart failure (NYHA class III and IV)

4) Current atrial fibrillation or Wolf-Parkinson-White (WPW) syndrome

5) Allergy to beta-blocker in subjects with resting heart rate > 65 bpm

6) Systolic blood pressure > 160 mm Hg

7) Diastolic BP > 100 mm Hg

8) History of asthma only if unable to tolerate beta-blockers

9) Allergy to iodinated contrast material or shellfish

10) Allergy to nitroglycerin

11) BMI > 35 kg/m² if female and > 40 kg/m² if male

12) Body weight > 350 lbs

13) Use of drugs for weight loss [e.g. Xenical (orlistat), Meridia (sibutramine), Acutrim (phenylpropanolamine) or similar over-the-counter medications] within three months of screening

14) Surgery within 30 days of screening

15) History of acquired immune deficiency syndrome or human immunodeficiency virus (HIV)

16) Poor mental function or history of dementia/Alzheimer’s Disease or on medications used for treatment of dementia [e.g. Tacrine (Cognex), Rivastigmine (Exelon), Galantamine (Razadyne, Reminyl), Donepezil (Aricept), Memantine (Namenda)] or any other reason to expect patient difficulty in complying with the requirements of the study
17) Medicine for erectile dysfunction within 72 hours prior to MDCTA

**Exclusions Specific to Salsalate Trial:**

1) Prior hemorrhagic stroke
2) persons with known aspirin allergy
3) History of type 1 diabetes and/or history of ketoacidosis
4) Use of continuous oral corticosteroid treatment (more than 2 weeks), or patients requiring corticosteroids within 3 months
5) Anti-diabetic medication including thiazolidinedione (pioglitazone or rosiglitazone), or insulin or Extendin-4 (Byetta)
6) History of peptic ulcer or gastritis within 5 years
7) Positive stool guaiac
8) Hemoglobin 2 standard deviations below normal
9) Low platelet count (2 standard deviations below normal)
10) Known bleeding disorder
11) Coumadin (warfarin compounds)
12) History of malignancy, except subjects who have been disease-free for greater than 5 years, or whose only malignancy has been basal or squamous cell skin carcinoma
13) History of drug or alcohol abuse, or current weekly alcohol consumption >14 units/week (1 unit = 1 beer, 1 glass of wine, 1 mixed cocktail containing 1 ounce of alcohol)
14) Daily use of NSAIDS (including salsalate) for arthritis
15) Use of probenecid (Benemid, Probalan), sulfinpyrazone (Anturane) or other uricosuric agents
16) Chronic, severe tinnitus (pt self report of 5/10 or greater intensity). *
17) History of gastric bypass surgery
18) Urine microalbumin/creatinine > 300 mcg/mg cr
19) History of significant chronic rheumatologic or other chronic inflammatory disease (including foot ulcers)

*Patients who report mild, chronic tinnitus (4/10 intensity or less) may be considered for participation

**Exclusions specific to Lifestyle Trial:**

1) Prior stroke with residual cognitive deficit or functional deficit preventing any type of exercise
2) Current chemotherapy or radiation for malignancy
3) Current weekly alcohol consumption > 21 units/week (1 unit = 1 beer, 1 glass of wine, 1 mixed cocktail containing 1 ounce of alcohol
CHAPTER 5: BENEFITS AND RISKS

5.1 Possible Benefits

Clinical research studies are not designed to provide individual subjects with health benefit from taking part in the study. It is our hope that the information we learn from this study will help us to better understand the role of inflammation in development of coronary artery plaque and new means to prevent plaque progression in high-risk patients. Additionally, much will be learned about a new, less invasive means to assess coronary artery disease.

However, patients may be found to have a significant coronary lesion of >70% in a proximal vessel that would be considered of very high risk and lead to medical intervention averting a serious and potentially life-threatening event.

Additionally, overweight/obese patients are also at risk of development of diabetes. Lifestyle modification, as proposed in this study could prevent progression to diabetes or improve metabolic control and risk of micro-vascular complications in patients with established disease. There are other health and personal benefits of weight management and physical fitness. Likewise, multiple small studies suggest salicylates may be beneficial in diabetes treatment or prevention. Subjects will undergo extensive health assessments that may lead to early detection or improved management of other disorders, thereby resulting in individual benefit.

5.2 Possible Risks and Analysis of Risk/Benefit Ratio

5.2.1 Lifestyle:

Subjects may be inconvenienced by the time commitment involved in participation in the study. Study subjects will have a graded exercise or pharmacological stress test within 12 months prior to visit 2 provided there has been no change in anginal pattern during that time. If they have had a change in anginal pattern, they need a new ETT. Dr. Welty will review all ETT results, calculate maximal target heart rate and provide exercise prescriptions for each study subject based on their ETT results. We will provide instructions that they not exceed 70-85% of their predicted maximal heart rate if they have no angina. If they have stable angina, they must not exceed 70% of the heart rate at which they developed angina during their stress test. For patients without angina during stress and with a reversible defect on nuclear imaging or an inducible wall motion abnormality during echocardiography without subsequent revascularization, exercise will be limited to 70% of the peak HR achieved during exercise.

5.2.2. Salsalate (Disalcid®):

Salicylates like aspirin are some of the most commonly used over the counter drugs. Salicylates are typically used for treatment of minor aches and pains as well as chronic inflammatory diseases like arthritis. The dose of salsalate proposed in this study is FDA approved for use in treatment of patients with chronic arthritis-type pains. Salicylates can cause transient nausea, anorexia, heartburn, and tinnitus rarely with some degree of deafness. These effects usually subside as treatment continues. The dose of salsalate will be reduced in 500 mg increments (to as low as 500 mg) for adverse effects, persistent discomfort or toxic serum levels, or subjects will be taken off study drug. Salsalate is not absorbed in the stomach and is associated with lower risks of gastrointestinal discomfort and blood loss than aspirin. Unlike other nonsteroidal anti-inflammatory drugs (NSAIDs), salsalate is a weak inhibitor of COX enzymes and does not prolong bleeding time. Blood levels of salicylate will be monitored during the study period to ensure therapeutic dosing. Levels greater than 30 (toxic dosing) will warrant dose reduction by 500 mg increments. A decrease in eCrCLCG to <50 ml/min will warrant dose reduction by 500 mg increment. Salsalate will be stopped for development of guiac positive stool or fall in hematocrit to < 2 standard deviations below the lower limit. Subjects could...
develop allergic reactions to the drug during treatment, although this is rare. Renal function will be monitored by use of estimated glomerular filtration rates and urinary albumin excretion.

**Black Box warning regarding the use of non-selective NSAIDS.** In January 2006 the FDA mandated the addition of a Drug Class Black Box Warning in the package insert of all NSAIDS but aspirin. The boxed warning is highlighting the potential for increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal, and further saying that the risk is greater in patients with cardiovascular disease or risk factors for cardiovascular disease. This requirement was based on the recent information regarding the potential for increased cardiovascular risk with the use of two selective COX-2 inhibitors Valdecoxib (Bextra) and Rofecoxib (Vioxx) that led to their withdrawal from the market. Aspirin is the only NSAID excluded form this demand due to its platelet inhibitory effect and the fact that it had been shown in clinical trials to reduce the risk of cardiovascular events. In contrast to most other NSAIDS, salsalate is a weak COX-1 and COX-2 inhibitor and therefore its potential to increase CVD risk is small. Moreover having been in clinical use for so many years without any reported excess cardiovascular events, we believe that salsalate is a safe drug also in that regard.

In the black box there is also a warning concerning increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. The issue of gastrointestinal risk mentioned in the black box is already addressed in the section about gastrointestinal risks above.

5.2.3 Placebo:

There are no known risks of placebo.

5.2.4 Multi-detector computed tomographic angiography:

**Contrast exposure:** MDCTA involves the use of contrast agents. Both minor and major reactions to contrast material can occur. Minor reactions to contrast material include pain at the injection site, skin rash with itchiness, facial flushing, nausea and eye swelling. More severe reactions can occur including asthma and a fall in blood pressure. The reported incidence of deaths from contrast material is approximately 2.1 per million exams. Leakage of the dye can occur around the catheter into the tissues at the site of the catheter causing skin bruising. A physician will be present throughout the course of the injection to monitor the study. Subjects will not be allowed to participate in this study with a history of past allergic reaction to contrast agents. Subjects with renal dysfunction are excluded at screening. Estimated glomerular filtration rate eCrCLCG must be ≥ 60 ml/min at study entry, and ≥ 55 ml/min at end of study evaluation. This will permit complete evaluation in most subjects as average rate of decline in renal function in this group can be expected to be about 0-2 ml/min/yr. For those few subjects who may have a Cr >1.3 mg/dL or CrCl < 55 ml/min, we will use the Beth Israel Deaconess Hospital clinical radiology protocol for hydration: Cr level 1.3 to 1.6 mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of Optiray 320 or 350. For Cr level of 1.7 to 1.9 mg/dL: oral hydration pre and post administration of 100cc of Visipaque. Subjects treated with metformin will have the medication held on the day of MDCTA and the day following the procedure.

**Beta Blocker:** MDCTA studies are technically limited if the heart rate exceeds 65 beats per minute. For subjects with a heart rate greater than this, the beta-blocker Metoprolol (50 mg) will be administered by mouth to reduce the rate to less than 65 beats per minute one hour before the MDCTA. If the subject’s heart rate remains greater than 65 beats per minute at 1 hour after 50 mg of metoprolol is given by mouth, additional metoprolol will be given per the current radiology protocol until the heart rate goes down to 65 beats per minute. This will be given
through an intravenous injection in the presence of a physician. Metoprolol can cause bradycardia (or slow heart rate) which could also reduce blood pressure. However, this dose of Metoprolol is not usually associated with light-headedness, severe slowing of the heart, nausea or vomiting. Furthermore, medical history and electrocardiograms will be obtained prior to MDCTA and Metoprolol will not be administered to patients with WPW (Wolff-Parkinson-White syndrome), bronchial asthma, impaired liver function, or kidney dysfunction as beta blockers such as Metoprolol may have adverse effects in persons with these conditions.

**Nitroglycerin:** Sublingual nitroglycerin (0.4 mg) will be given to make the coronary arteries more prominent on MDCTA. This is standard procedure during MDCTA. Rarely, this causes a headache. The use of medications to improve erectile dysfunction (Viagra, Levitra, Cialis) in conjunction with nitroglycerin can cause severe drop in blood pressure and shock. Therefore subjects will be instructed not take these medications for 72 hours prior to the MDCTA testing. Subjects with known allergy to nitroglycerin will not be able to participate in the study.

**Radiation exposure:** This research protocol involves exposure to radiation from two angiographic CT scans, one at baseline and the other at the final visit 30 months later. The amount of radiation exposure subjects will receive is equivalent to a whole body exposure of 4.8 rem. This is equivalent to 108% of the annual radiation exposure limit allowed for a radiation worker such as the technologist performing the CT scan (and 43% of the technologist exposure limit for 30 months). The risk from radiation exposure of this magnitude is considered to be comparable to other every day risks.

**5.2.5 Blood Loss:**

The total amount of blood taken for the study is approximately 450 ml. This is about one pint of blood typically given at one time at a blood bank but it is distributed over the entire 30 month study and should not pose significant risk to the health of the participant. Subjects will be advised not to donate blood for two months before or after participation in the study. Subjects with anemia (hemoglobin 2 standard deviations below normal) will be excluded from participation.

**5.2.6 Phlebotomy, intravenous catheters:**

These procedures may be associated with local pain, bruising, bleeding and infection. Rarely, nerve damage or phlebitis may develop at the puncture site. These complications usually resolve spontaneously or with local heat application. Subjects may be inconvenienced by the time commitment involved in participation in the study. There may be other risks from this study not yet identified. No psychological, social or legal risk is anticipated. These risks are addressed in the protocol and will be described to participants in the consent form documents.

**5.2.7 Data Protection and safety:**

All study staff will be trained and certified in privacy protection, the Health Insurance Portability and Accountability Act (HIPAA), and the ethical conduct of clinical research. We cannot fully protect patient confidentiality in this study as patient registration at the Beth Israel Deaconess Medical Center (BIDMC), radiology procedures, and safety laboratories at Quest Diagnostic Laboratories are all linked to patient name. However, persons working in BIDMC registration, radiology department, as well as other professionals who may come in contact with identifiable information are professionally trained to respect this sensitive information. The same holds true for professionals at Quest Diagnostics, which operates under a Massachusetts State License. Identifiable patient information, i.e. patient name, will also be used to communicate with the patient/subject’s cardiologist. However, the site provider(s) is/are also aware of the private
information. Use of identifiable information is essential to facilitate communication with study personnel directly involved in patient follow-up, especially as sites use different medical record numbers than either the BIDMC or the study specific ID.

The results from Quest Diagnostic Laboratories will be transferred through the secure, protected data stream existing between Quest and the BIDMC which is used and approved for the transfer of clinical information. The medical and laboratory data will be maintained though our data coordinating center such that individual data will be available only to the steering committee, medical monitor and data coordinating center, and providers directly caring for the individual study subject using a password protected system.

Results of the MDCTA will likewise be stored through the trial specific secure electronic medical data system with access restricted to the steering committee members, the medical monitor, the data coordinating staff, and the study staff who are directly involved in the care of the individual patient using the same password protection system.

Names, social security numbers and other types of personal identification will not appear on study forms, data files or materials sent to anyone outside the approved local study site staff. All study subjects’ data and specimens shared with other bodies (including the research core laboratory) will be identified only by their coded study ID with no personal identifiers. The link between blood samples and the individual that it belongs to will be available only to the individual care provider, the steering committee and the data coordinating center.

Access to the study website will be granted only to pre-authorized study staff using a stringent personal password. Each site will have access only to their site patients’ information. Solely the steering committee, the study medical monitor and the data coordinating center will have access to all study patients information. Individual identifiers will be removed from records prior to data assembly and statistical analysis, and only the code ID’s will be used. No subject will be identified in any publication from this study.

All study forms and materials collected for the study are stored in secure, locked locations. Each study site will maintain a file on each patient that includes personal identifiers, including contact information linked to the participant coded study ID number. These data are not transferred to the Administrative Core. The files are kept in secure locations and the study site is responsible for taking every other reasonable measure (those set by the state, the site, and the study) to ensure and maintain record confidentiality and patient privacy.
CHAPTER 6: DATA SAFETY MONITORING PLAN

Lifestyle Trial and Study Principal Investigator Name: Francine Welty, MD, PhD
And Lifestyle trial Co-PI: Dr. Ernst Schaefer.

Salsalate Trial Principal Investigator Name: Dr. Allison B. Goldfine, MD

Overview of DSMP

Brief description of the purpose of the study. The overall goal of this project is to compare the effect of salsalate and an intensive lifestyle intervention geared to weight loss to usual care/placebo on regression or progression of soft plaque in overweight/obese persons with coronary artery disease.

Adherence statement. The Data Safety Monitoring Plan (DSMP) outlined below for this application will adhere to the protocol submitted to and approved by the Human Subject Committees of each participating center.

Data quality and management. The principal investigator will review all data collection forms on a bi-monthly basis for completeness and accuracy of the data as well as protocol compliance.

6.1 The person/process by which the data will be analyzed for subject safety issues

Dr. Welty and Schaefer are co-principal investigators of the lifestyle arm and of the trial in total. Dr. Goldfine is the principal investigator of the salsalate and placebo arms of the trial and the site responsible investigator at the Joslin Diabetes Center.

6.1.1 Safety reviews

The project leader for lifestyle trial (Welty) and her co-PI Dr. Schaefer and study coordinator will oversee study patient safety and review the safety and progress of the lifestyle trial on a bi-monthly basis. Dr. Goldfine, will be the responsible principal investigator for salsalate trial and will oversee study patient safety also bi-monthly. Safety labs will be reviewed as they come in immediately by the research team for each respective project. Any abnormal labs will then be reviewed immediately with the respective project leader. Reports for any adverse event of moderate or greater severity will be generated by the research team for each project and reported to the Principal Investigators (Dr. Francine Welty and Dr. Allison Goldfine).

6.1.2 Site reviews

We propose a monitoring plan that satisfies the Guideline for Monitoring of Clinical Investigations of the Food and Drug Administration working in collaboration with the Institutional Review Board Committees, including both technical and scientific reviews once a year. The study coordinator or research nurse for each project will ensure collection of data from the clinical sites and review all materials for complete collection.

*Regulatory Document and Informed Consent Review: The study coordinator or research nurse would conduct a review of 100% of the informed consents to assure proper procedures were followed, as well as 100% review of the regulatory documents to assure that all required/critical documents were present. There would be immediate verbal and written feedback provided to the site. Sites providing incomplete documentation will be visited by a member of the study team. During the site visit, there will also be immediate verbal feedback provided and a written report would follow and would include a detailed itemization of discrepancies and items
requiring follow-up or reconciliation. This report would also be forwarded to the Principal Investigator, site Investigator, and Institutional Review Committee.

6.1.3 Annual reviews

The Project Leaders will review their respective project protocol on a continuing basis for subject safety and site responsible investigators will provide written summary of results annually for review in the annual progress reports submitted to the Principal Investigator (Dr. Welty), safety officer, GCRCs, IRBs and NHLBI.

6.1.4 Annual reports

The annual report will include a list of adverse events. These will be provided to the NIH and institutional review boards.

6.1.5 Content of annual reports

The annual report will address: (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely.

6.1.6 GCRC reviews

The GCRC Research Review Committee will also review each protocol annually for safety.

6.1.7 Laboratory review

Laboratory assessment will be performed at scheduled and predetermined intervals. The research team will review all laboratory reports within 24 hours of completion by the laboratory (Quest Diagnostics). Laboratory reports will be provided to the medical care provider. The data monitor will review laboratory profiles monthly for unexpected trends and will bring any such trends to the attention of the Data Safety Monitoring Board for consideration of need for early study termination.

6.2 Rules for stopping the trial in the event that expected or unexpected adverse events arise

Coronary Artery Disease and Exercise: If subjects develop adverse events (unstable angina or other CVD events in association with exercise), they will need to be treated appropriately by their treating physicians (medication or revascularization). If they have a repeat ETT, which is acceptable and once their MD feels they can resume exercise, they can resume their exercise program. If they cannot exercise, they will continue to follow dietary recommendations and take Lovaza, and we will continue to follow them. They will not exit the study.

We will provide a manual with instructions to call primary doctor and study coordinator when these events occur. We will request a contact phone number (other than primary doctor) on their contact information sheet. Subjects are scheduled to see their Cardiologist at 6, 12, 18 and 24 months after initiation of the study drug. Medical history, review of system, and physical examination reports will be reviewed by a member of the study team and used to capture adverse events. Discharge summaries from all hospital admissions will be obtained, with permission provided at the time of the baseline evaluation. In addition subjects will speak by phone to a member of the study team at scheduled intervals, specifically 6 weeks, 3, 6, 9, 12, 18, and 24 months for potential adverse events.
Subjects who do not show up for a scheduled visit or are unavailable for a scheduled phone call will be tracked in multiple step-wise ways including calling the individual provided by the subject to be contacted in event of emergency, calling the primary care physician, checking for obituary listings, public death records (the Social Security Administration death index available at www.Ancestry.com); and finally checking the National Death Index if none of the other methods identify the status of the subject. Additionally, scheduled shipments of study medications will be terminated for subjects who do not show up for scheduled visits.

We have provided a mortality endpoint algorithm. We have an endpoint manual for cardiovascular events, which we have provided. We also have algorithms for unstable angina, hospitalized angina, hospitalized CHF, first diagnosis/procedure for peripheral arterial vascular disease, coronary revascularization procedure, TIA, stroke and mortality. If they develop a newly diagnosed medical illness that would have excluded them from study participation, this will be reported. All events will be reported to the DSMB.

6.2.1 Adverse Events

6.2.1.1 Adverse event grading

Attribution scale

Adverse event will be defined as either an expected side effect that is of a serious nature, or an unexpected side effect/event regardless of severity. Adverse events occurring during study participation will be assessed for severity (mild, moderate, severe or life threatening) by a site responsible study investigator and related to study drug or procedure (unrelated, unlikely, possibly, probably or definitely), and all events will be graded as to their attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol). Any event that is reported to either the site responsible investigator or the designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented as such. All actions taken will be recorded and outcomes to adverse events assessed.

BIDMC uses the adverse event definitions listed on the FDA website: (www.access.gpo.gov/nara/cfr)

(21cfr312.32) All adverse events must be reported to both the CCI and the RSSO according to CCI guidelines.

The BIDMC website for adverse event reporting guidelines is: http://research.caregroup.org/OST/ClinicalTrials/AEGuidelines.asp

We will use the FDA definitions of Adverse Events as follows:

a) **Adverse Event (AE).** Any event that occurs during a clinical study, which results in the subject experiencing a new symptom or a worsening of an already existing symptom.

b) **Disability.** A substantial disruption of a person's ability to conduct normal life functions.

c) **Serious Adverse Event (SAE).** Any adverse drug experience occurring during a clinical trial that results in any of the following outcomes:

- Death
- Life-threatening event
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect
• Development of cancer
• Drug overdose
  also
• Important medical or psychological events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the above outcomes.

d) **Unexpected Adverse Event.** Any adverse experience not identified in nature, severity or frequency in the investigator’s drug brochure or included in the risk information described in the protocol or the informed consent.

c) **Moderate Adverse Event.** Discomfort severe enough to cause interference with usual activities; persistent or requiring treatment.

f) **Mild Adverse Events.** Awareness of signs or symptoms, but easily tolerated; are of minor irritant type; causing no loss of time from normal activities; symptoms would not require medication or a medical evaluation; signs and symptoms are transient.

6.2.1.2 Reporting of severe or life threatening adverse events

Severe or life threatening adverse events will be reported to the CCI, GCRC and DSMB within 24 hours. The CCI, GCRC and/or DSMB may stop the trial for severe or life threatening events that are probably or definitely related to the study drug or procedures.

6.2.1.3 Plan for reporting both anticipated and unanticipated adverse events

Each subject is evaluated for any adverse events. Any event that is reported to either the investigator or the designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented as such. Any event that is reported will then generate an adverse event report, which will be submitted to the principal investigator, the data manager and the multiple Institutional Review Boards and Clinical Research Centers. The report will include a description of the event, when and how it was reported, as well as any official chart records or documentation to corroborate the event or the reporting of the event. All adverse events will be graded as mild, moderate, or severe. Any severe and/or unanticipated adverse event will be immediately reported to the investigators, data monitor, institutional safety officers, multiple Institutional Review Boards and Clinical Research Centers. All other adverse events will be reported to the safety officer, multiple Institutional Review Boards and Clinical Research Centers, preferably within 2 weeks of the date of the event. All adverse events will be summarized annually and submitted to the multiple Institutional Review Boards and Clinical Research Centers. Any action resulting in a temporary or permanent suspension of this study (e.g. FDA actions, IRB actions, or actions by a commercial sponsor or by the investigators or co-investigators) will be reported to the appropriate NHLBI program official.

6.2.1.4 Expected risks:

**Exercise:** Study subjects will have a graded exercise or pharmacological stress test within 12 months prior to visit 2 provided there has been no change in anginal pattern during that time. If they have had a change in anginal pattern, they need a new ETT. Dr. Welty will review all ETT results, calculate maximal target heart rate and provide exercise prescriptions for each study subject based on their ETT results. We will provide instructions that they not exceed 70-85% of their predicted maximal heart rate if they have no angina. If they have stable angina, they must not exceed 70% of the heart rate at which they developed angina during their stress test. For
patients without angina during stress and with a reversible defect on nuclear imaging or an inducible wall motion abnormality during echocardiography without subsequent revascularization, exercise will be limited to 70% of the peak HR achieved during exercise.

**Lovaza:** Lovaza, a form of pharmacologic grade fish oil, is generally well tolerated and no serious reactions have been reported. However, belching with a fishy odor, upset stomach, and changes in taste have been reported along with other side effects that have not been clearly related to fish oil pills (these include infection, flu, back pain, and chest pain). If study subjects have difficulty tolerating the fish oil capsules, the dose will be reduced to two per day. If difficulty continues, fish oil will be stopped. The study subject will continue in the study. Lovaza is a prescription medication available in the US only with a physician’s prescription.

**Salsalate (Disalcid®):** Salicylates, like aspirin, are some of the most commonly used over the counter drugs. Salicylates are typically used for treatment of minor aches and pains as well as chronic inflammatory diseases like arthritis. The dose of salsalate proposed in this study is used in treatment of patients with chronic arthritis-type pains. Salicylates can cause transient nausea, anorexia, heartburn, and tinnitus rarely with some degree of deafness. These effects usually subside as treatment continues. The dose of salsalate will be reduced in 500 mg increments (to as low as 500 mg) for adverse effects, persistent discomfort or toxic serum levels, or subjects will be taken off study drug. Salsalate is not absorbed in the stomach and is associated with lower risks of gastrointestinal discomfort and blood loss than aspirin. Unlike other nonsteroidal anti-inflammatory drugs (NSAIDs), salsalate is a weak inhibitor of COX enzymes and does not prolong bleeding time. Blood levels of salsalate will be monitored during the study period to ensure therapeutic dosing. Levels greater than 30 (toxic dosing) will warrant dose reduction by 500 mg increments. A decrease in eCrCLCG to < 50 ml/min will warrant dose reduction by 500 mg increments. In addition to the increased frequency of assessing urinary microalbumin, and in accordance with the recommendations of the data safety monitoring board for this study, any participant who had a baseline MCR < 150 mcg/mg cr, who develops a microalbumin to creatinine ratio (MCR) of 300 mcg/mg cr or greater will have the laboratory assessment repeated with an unscheduled visit and for sustained elevation be evaluated by a nephrologist. For participants who entered the study with a baseline MCR > 150 mcg/mg cr we will recommend a nephrology consultation for a sustained doubling of the MCR rather than using a set cutoff value. Additionally, for participants with a large change, as defined above, discontinuation of the study drug will be strongly considered. All attempts will be made to keep the subject in the trial and evaluate the renal function after study drug discontinuation. Salsalate will be stopped for development of guaiac positive stools or fall in hematocrit to < 2 standard deviations below the lower limit. Subjects could develop allergic reactions to the drug during treatment and although rare, allergic reactions will warrant early termination.

**Placebo:** There are no known risks of placebo.

**Multi-detector computed tomographic angiography:**

**Contrast exposure:** MDCTA involves the use of contrast agents. Both minor and major reactions to contrast material can occur. Minor reactions to contrast material include pain at the injection site, skin rash with itchiness, facial flushing, nausea and eye swelling. More severe reactions can occur including asthma and a fall in blood pressure. The reported incidence of deaths from contrast material is approximately 2.1 per one million exams. Leakage of the dye can occur around the catheter into the tissues at the site of the catheter causing skin bruising. A physician will be present throughout the course of the injection to monitor the study. Subjects will not be allowed to participate in this study with a history of past allergic reaction to contrast agents. Subjects with renal dysfunction (eCrCLCG < 60 ml/min) are excluded at screening. For those few subjects who may have a Cr > 1.3 mg/dL or CrCl < 55 ml/min, we will use the Beth
Israel Deaconess Hospital clinical radiology protocol for hydration:  Cr level 1.3 to 1.6 mg/dL: oral hydration (1 liter of water by mouth) pre and post administration of Optiray 320 or 350.  For Cr level of 1.7 to 1.9 mg/dL: oral hydration pre and post administration of 100cc of Visipaque. Subjects treated with metformin will have the medication held on the day of MDCTA and the day following the procedure.

**Beta Blocker:** MDCTA studies are technically limited if the heart rate exceeds 65 beats per minute. For subjects with a heart rate greater than this, the beta-blocker Metoprolol (50 mg) will be administered by mouth to reduce the rate to approximately 60 beats per minute one hour before the MDCTA. If the subject’s heart rate remains greater than 65 beats per minute at 1 hour after 50 mg of metoprolol is given by mouth, 5 mg metoprolol will be given every 5 minutes up to 3 times or until the heart rate goes down to 65 beats per minute. This will be given through an intravenous injection in the presence of a physician. Metoprolol can cause bradycardia (or slow heart rate) which could also reduce blood pressure. However, this dose of Metoprolol is not usually associated with light-headedness, severe slowing of the heart, nausea or vomiting. Furthermore, medical history and electrocardiograms will be obtained prior to MDCTA and Metoprolol will not be administered to patients with WPW (Wolff-Parkinson-White syndrome), bronchial asthma, impaired liver function, or kidney dysfunction as beta blockers such as Metoprolol may have adverse effects in persons with these conditions.

**Radiation exposure:** This research protocol involves exposure to radiation from two angiographic CT scans, one at baseline and the other at the final visit 30 months later. The amount of radiation exposure subjects will receive is equivalent to a whole body exposure of 4.8 rem. This is equivalent to 108% of the annual radiation exposure limit allowed for a radiation worker such as the technologist performing the CT scan (and 43% of the technologist exposure limit for 30 months). The risk from radiation exposure of this magnitude is considered to be comparable to other every day risks.

**Nitroglycerin:** Sublingual nitroglycerin (0.4 mg) will be given to make the coronary arteries more prominent on MDCTA. This is standard procedure during MDCTA. Rarely, this causes a headache. The use of medications to improve erectile dysfunction (Viagra, Levitra, Cialis) in conjunction with nitroglycerin can cause severe drop in blood pressure and shock. Therefore subjects will be instructed not take these medications for 72 hours prior to the MDCTA testing. Subjects with known allergy to nitroglycerin will not be able to participate in the study.

**Blood Loss:** The total amount of blood taken for the study is approximately 450 ml. This is about one pint of blood typically given at one time at a blood bank, but it is distributed over the entire 30 month study and should not pose significant risk to the health of the participant. Subjects will be advised not to donate blood for two months before or after participation in the study. Subjects with anemia (hemoglobin 2 standard deviations below normal) will be excluded from participation.

**Phlebotomy, intravenous catheters:** These procedures may be associated with local pain, bruising, bleeding and infection. Rarely, nerve damage or phlebitis may develop at the puncture site. These complications usually resolve spontaneously or with local heat application. Subjects may be inconvenienced by the time commitment involved in participation in the study. There may be other risks from this study not yet identified. This study addresses pharmacologic targets in the treatment of diabetes and no psychological, social or legal risk is anticipated. These risks are addressed in the protocol and will be described to participants in the consent form documents.

6.3 planned frequency of the data summary of the adverse events
a) By time—all information including medical history, physical examination and laboratory assessment will be assessed by a study investigator as collected or completed by the laboratory. Summary data (blinded) will be reviewed bi-monthly by the respective project leaders and reported to the study Principal Investigators (Dr. Welty and Dr. Goldfine). Reports will be provided to oversight committees - CCI, Joslin CHS, GCRC and DSMB - annually or more frequently upon request.

b) By number of subjects—no specific reporting will be provided based on number of subjects

c) By adverse event [AE]—of severe or more severe than moderate nature will be reported to the DSMB. This will permit the DSMB to terminate the trial early for unexpected risk. This will include all known cardiovascular events.

In addition we will specifically:

a) Report unexpected fatal or life-threatening adverse events to the CCI [617-667-0469] Joslin CHS, and the GCRC RSSO by phone [617-667-1764], fax [617-667-5953] or e-mail within one [1] business day, and provide a formal written report within seven [7] calendar days.

b) Report all other unexpected serious adverse events to the CCI Joslin CHS, and the GCRC RSSO within seven [7] calendar days.

c) Report expected serious adverse events to the CCI Joslin CHS, and the GCRC RSSO as soon as possible but within fourteen [14] calendar days.

d) Report unexpected, non-serious (moderate) adverse events in writing to the CCI Joslin CHS, and the GCRC RSSO within fourteen [14] calendar days.

e) The PI will provide the GCRC RSSO with any copies of adverse event reports that are sent to any entity such as the CCI, Joslin CHS, FDA, NIH, etc. within the same time constraints.

f) The PI will also provide the GCRC RSSO with an annual summary of adverse events (including anticipated, non-serious AEs), delineated by category and number.

In addition, for this multi-center research trial, adverse events at sites other than BIDMC or its official satellites will also be reported according to the above detail. Death, serious, or life-threatening events at other sites will be reported as noted above immediately upon learning of their occurrence.

6.4 Data Safety Monitoring Board

This study has a central Data Safety Monitoring Board (DSMB) convened by NHLBI in July, 2006. The central DSMB will review the study on an ongoing basis to ensure no new information is present to cause early study termination. The DSMB will convene 4 times a year. The names and position of the DSMB are:

DSMB chair: W. Tim Garvey, M.D., University of Alabama Birmingham

Executive Secretary: H. Eser Tolunay, Ph.D., NHLBI, NIH

Members: John D. Brunzell, M.D., University of Washington

Diane Catellier, DrPH, UNC-Chapel Hill

Mark Fillinger, M.D., Dartmouth-Hitchcock Medical Center
Ira J. Goldberg M.D., Columbia University Medical Center
Carl Grunfeld M.D., Ph. D., VA Medical Center San Francisco
Lisa Cooper Hudgins M.D., The Rogosin Institute/Rockefeller University
João A.C. Lima M.D., John Hopkins University
Howard Mann M.D., The University of Utah
Literature Cited


