(Anti-) Inflammation in T2D and CVD: Pathogenic Mediator and Clinical Outcomes

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Die Berliner Klinische Wochenschrift erscheint jeden Montag in der Stärke von wenigstens 1½ Bogen gr. 4. Preis vierteljährlich 6 Mark. Bestellungen nehmen alle Buchhandlungen und Post-Anstalten an.

Beiträge wolle man portofrei an die Redaction (N. W. Dorotheenstr. 78., 79.) oder an die Verlagsbuchhandlung von August Hirschwald in Berlin (N. W. Unter den Linden 68.) einsenden.

BERLINER KLINISCHE WOCHENSCHRIFT.

Organ für practische Aerzte.

Mit Berücksichtigung der preussischen Medicinalverwaltung und Medicinalgesetzgebung nach amtlichen Mittheilungen.

Redacteur: Prof. Dr. L. Waldenburg. Verlag von August Hirschwald in Berlin.

Montag, den 14. Juni 1876. № 24. Dreizehnter Jahrgang.

I. Zur Therapie des Diabetes mellitus, insbesondere über die Anwendung des salicylsauren Natron bei demselben.

Wilhelm Ebstein,
Professor in Göttingen.

In dieser Wochenschrift (Jahrg. 1873 No. 49, und 1875 No. 5) haben Julius Müller und ich den Nachweis geliefert, dass die Carbolsäure in einer Reihe von Fällen beim Diabetes mellitus die diabetischen Symptome zum Verschwinden bringt. Der Kreis unserer Beobachtungen hat sich in dieser Beziehung seit unserer letzten Publication bedeutend erweitert, und wir gedenken auf diesen Gegenstand in nächster Zeit wieder zurückzukommen, bei welcher Gelegenheit wir auch eine Reihe einschlägiger fremder positiver und negativer Erfahrungen mittheilen werden.

Ich schicke diese Bemerkung voraus, damit es nicht den Anschein habe, als solle durch die nachfolgenden Mittheilungen die günstige Wirkung der Carbolsäure bei gewissen, leider zur

wollen, in den Harnen, wo sich Salicylsäure nach dem Gebrauch von salicylsaurem Natron in so grosser Menge auffinden liess, durch Destillation des mit Salzsäure*) versetzten Harns, auch nur eine Spur von Carbolsäure durch Bromwasser im Ueberschuss im Destillat nachzuweisen. Jedoch will ich mich in diese Fragen heut nicht weiter vertiefen. Diese Zeilen sollen nur kurz die Geschichte zweier Fälle von Diabetes mellitus umfassen, bei denen die Carbolsäure und andere therapeutische Agentien einen unzureichenden Erfolg hatten, und wo beim ersten derselben das salicylsaure Natron die diabetischen Symptome vollkommen beseitigte, während es dieselben bei dem zweiten Kranken bedeutend besserte.

Eine genauere Ausführung der Fälle behalte ich mir für später vor. Diese Mittheilung soll eben die Collegen nur zu weiteren Versuchen in der besagten Richtung anregen.

1. Fall. Ludw. Freckmann aus Göttingen, Bürstenmacher, 58 Jahr alt, kam in Behandlung am 26. December 1875. Bis

Die Berliner Klinische Wochenschrift erscheint jeden Montag in der Stärke von wenigstens 1½ Bogen gr. 4. Preis vierteljährlich 6 Mark. Bestellungen nehmen alle Buchhandlungen und Post-Anstalten an.

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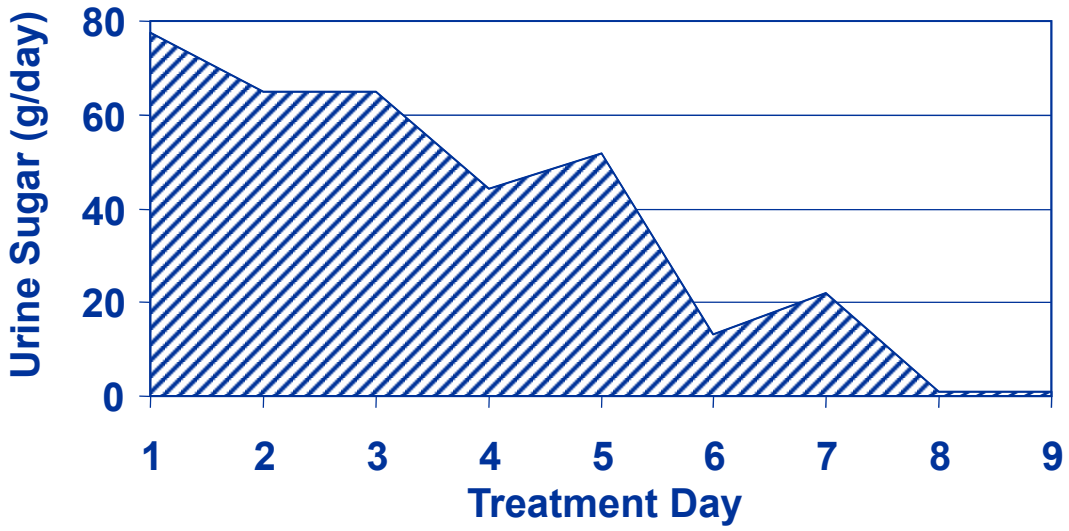


ON THE TREATMENT OF GLYCOSURIA AND DIABETES MELLITUS WITH SODIUM SALICYLATE.

By R. T. WILLIAMSON, M.D.Lond., F.R.C.P.
Physician to the Ancoats Hospital, Manchester, and Assistant Lecturer on Medicine, Owens College

IT is somewhat difficult to form a correct estimate of the action of drugs on the sugar excretion in cases of glycosuria and diabetes for many reasons. The occurrence of complications, such as phthisis for example, in the course of the disease may cause a diminution of the sugar excretion, which at first sight appears to be the result of medical treatment. Also in the treatment of diabetes the diet is restricted, more or less, at the same time that various drugs are prescribed; hence it is often impossible to say how much of the improvement which follows is due to the action of of the restricted diet and how much to the drug. In the most severe form of diabetes it is usually easy to demonstrate that no drug has much influence on the sugar excretion. But in the milder forms of diabetes or persistent glycosuria, considerable care is necessary in

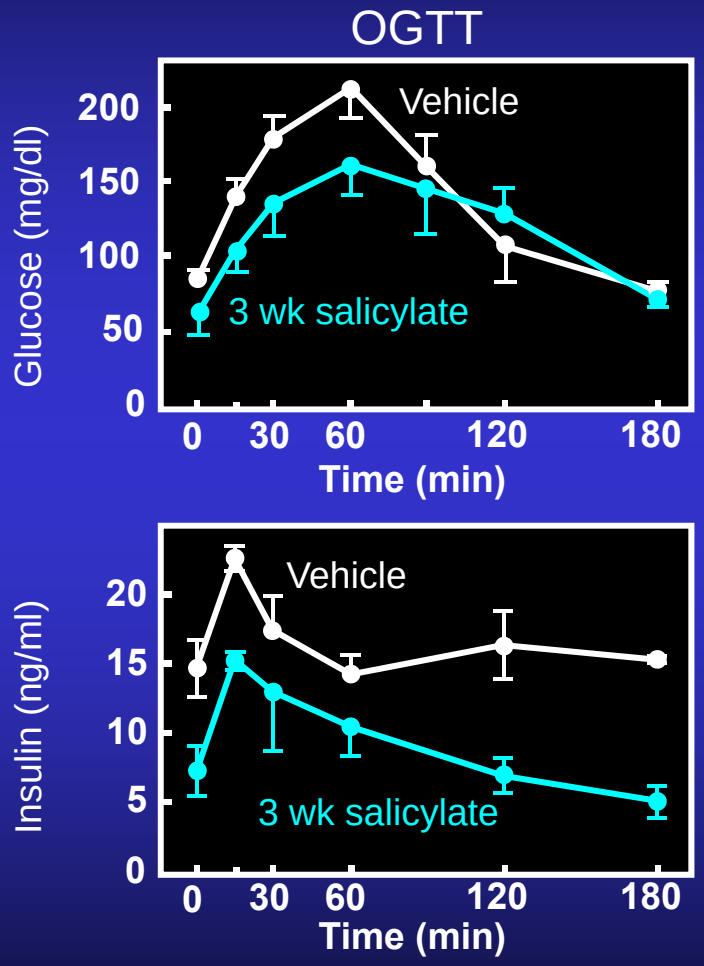
Second Period. -- For fifteen days the action of heroin or uranium nitrate was tried. The doses were small and only a slight diminution of the urine and the sugar excretion was observed during this period. The daily amount of urine was from 56 to 64 ounces; specific gravity, 1030 to 1038; amount of sugar, 22 grs. to 32 grs. to the ounce, and the daily excretion was 1,150 grs. to 1680 grs.

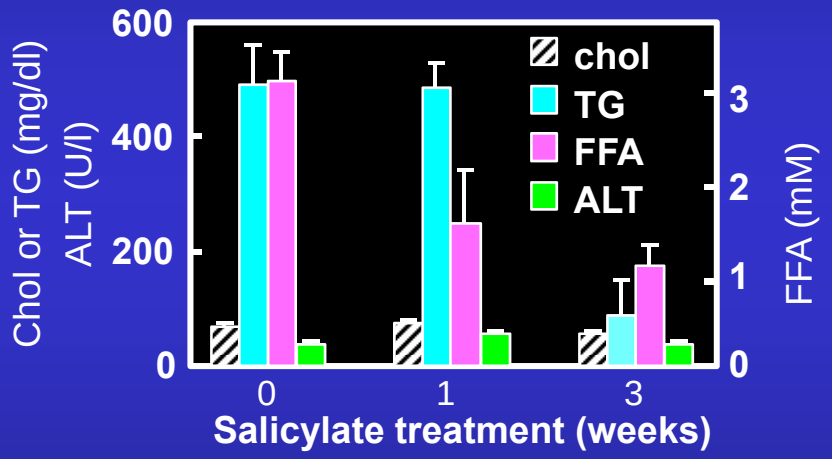
Third Period. -- Sodium salicylate was now commenced (on the twenty-sixth day after the patient was admitted into the hospital). At first 10 grs. were given three times a day, then four, five and six times a day; afterwards 15 grs. were given four times a day. The sugar excretion steadily diminished, and at the end of twenty-seven days the amount of urine was 43 ounces, the specific gravity, 1029; the sugar excretion, 10 grs. to the ounce, and 430 grs. in twenty-four hours.

Fourth Period. -- In addition to the 15 grs. of sodium salicylate given four times a day, salicylate of bismuth was now given as a powder for seventeen days. Sodium salicylate was now commenced (on the twenty-sixth day after the patient was admitted into the hospital). At first 10 grs. were given three times a day, then four, five and six times a day; afterwards 15 grs. were given four times a day.

"The results just recorded appear to me to prove conclusively that in this case sodium salicylate had a definite influence in greatly diminishing the sugar excretion."

High-Dose Salicylate in Zucker Fatty Rats





Yuan…Shoelson (2001) Science 293, 1673

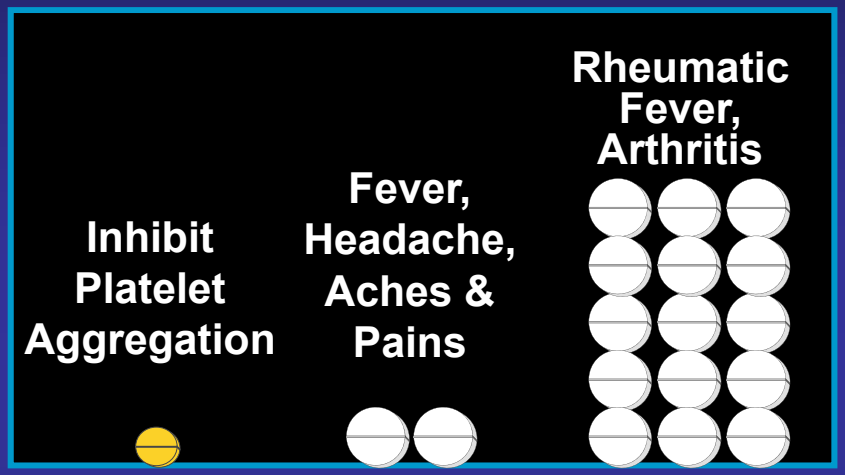
Do salicylates lower blood glucose and thus thus provide:

1) Clues to better understand molecular pathogenesis in insulin resistance, T2D and CVD?

2) Leads for pharmacological target identification and validation?

3) Potential new treatment strategies for patients with diabetes and CVD?

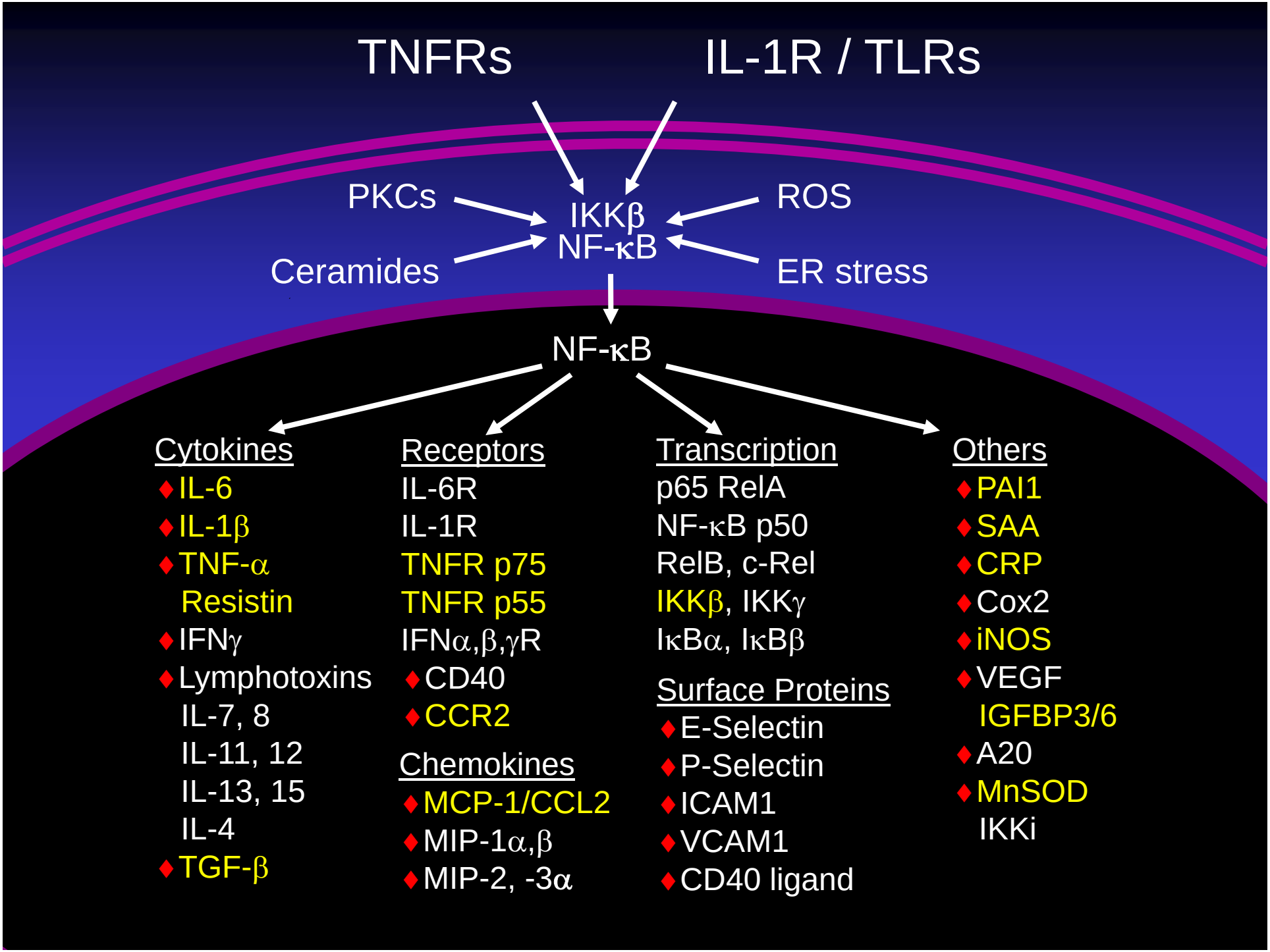
What is the Molecular Target of High-Dose Salicylate?



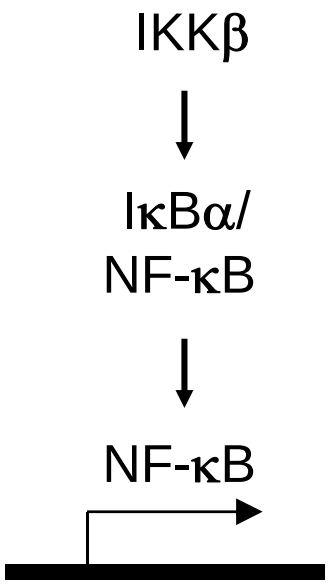
Kopp & Ghosh,
Inhibition of NF-κB by sodium salicylate and aspirin.
Science *265*, 956-959 (1994).

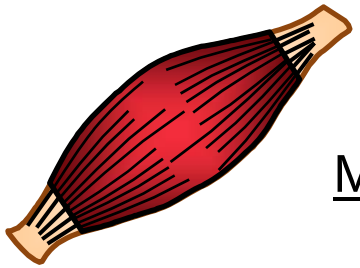
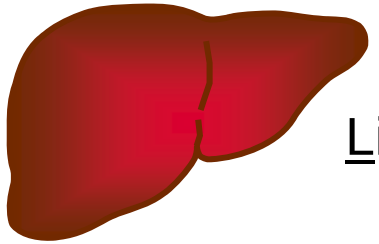
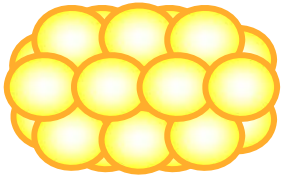
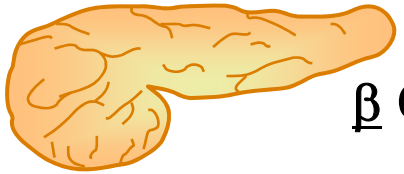
Yin, Yamamoto & Gaynor,
The anti-inflammatory agents aspirin and salicylate inhibit the activity of IkB kinase-β.
Nature *396*, 77-80 (1998).

Yuan, Konstantopoulos, Lee, Hansen, Li, Karin & Shoelson, Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of *Ikk*β. Science *293*, 1673-1677 (2001).

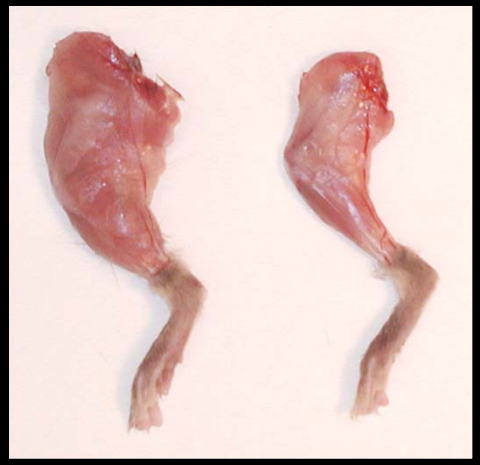

TNFRs
IL-1R / TLRs
PKCs
ROS
IKKβ
NF-κB
Ceramides
ER stress
NF-κB
Cytokines
♦IL-6
♦IL-1β
♦TNF-α
Resistin
♦IFNγ
♦Lymphotoxins
IL-7, 8
IL-11, 12
IL-13, 15
IL-4
♦TGF-β
Receptors
IL-6R
IL-1R
TNFR p75
TNFR p55
IFNα,β,γR
♦CD40
♦CCR2
Chemokines
♦MCP-1/CCL2
♦MIP-1α,β
♦MIP-2, -3α
Transcription
p65 RelA
NF-κB p50
RelB, c-Rel
IKKβ, IKKγ
IκBα, IκBβ
Surface Proteins
♦E-Selectin
♦P-Selectin
♦ICAM1
♦VCAM1
♦CD40 ligand
Others
♦PAI1
♦SAA
♦CRP
♦Cox2
♦iNOS
♦VEGF
IGFBP3/6
♦A20
♦MnSOD
IKKi

What are the Primary Sites of IKKβ/NF-κB Mediated Insulin Resistance?

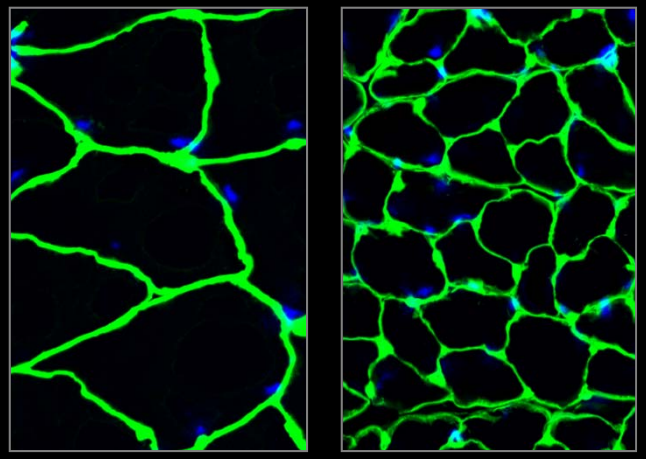


	Activated IKKβ SS/EE	Inhibited IκBα SR
 Muscle, MCK	MIKK	MISR MIKK-KO
 Liver, Alb	LIKK	LISR
 Fat, aP2	FIKK	FISR FIKK-KO
 β Cell, Ins2	βIKK	βISR βIKK-KO

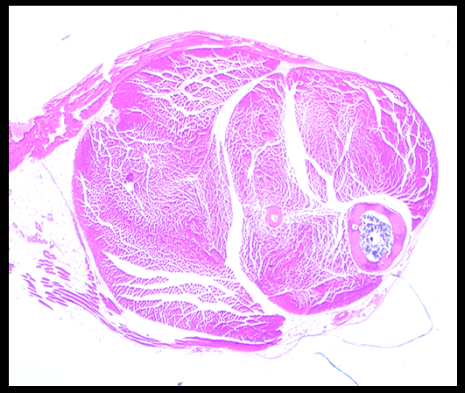
Cai et al., Cell 119, 285 (2004); Nat Med 11,183 (2005)

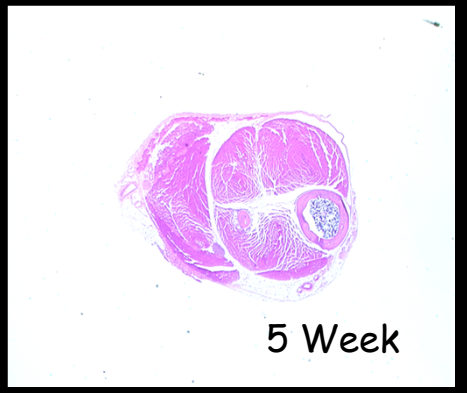


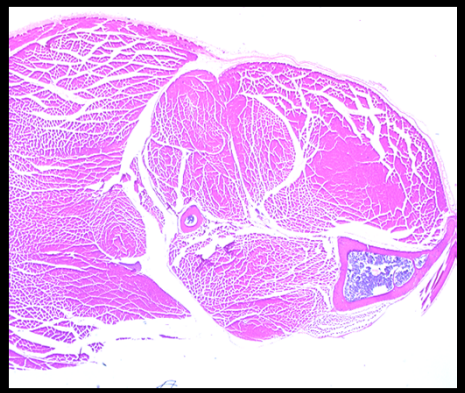
WT MIKK

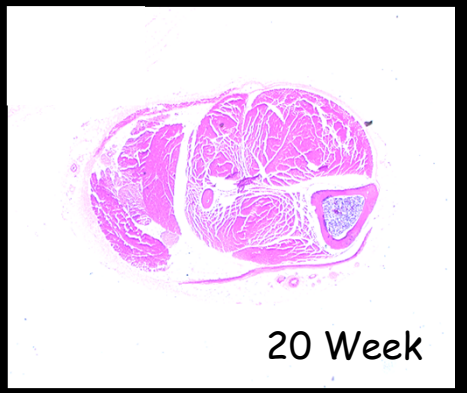


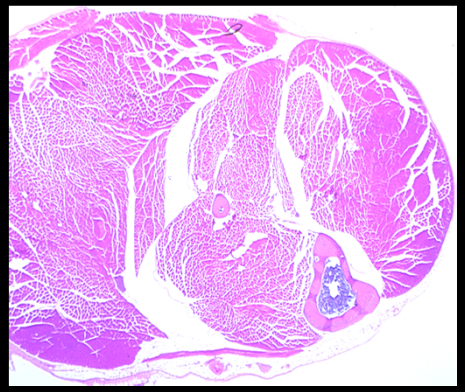
WT MIKK

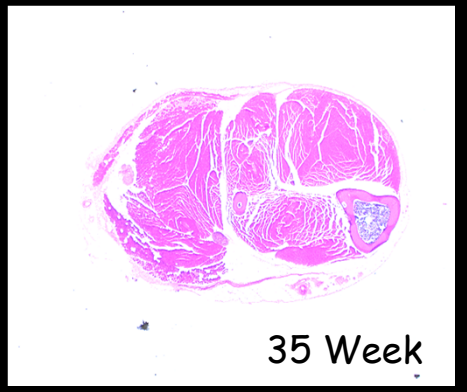




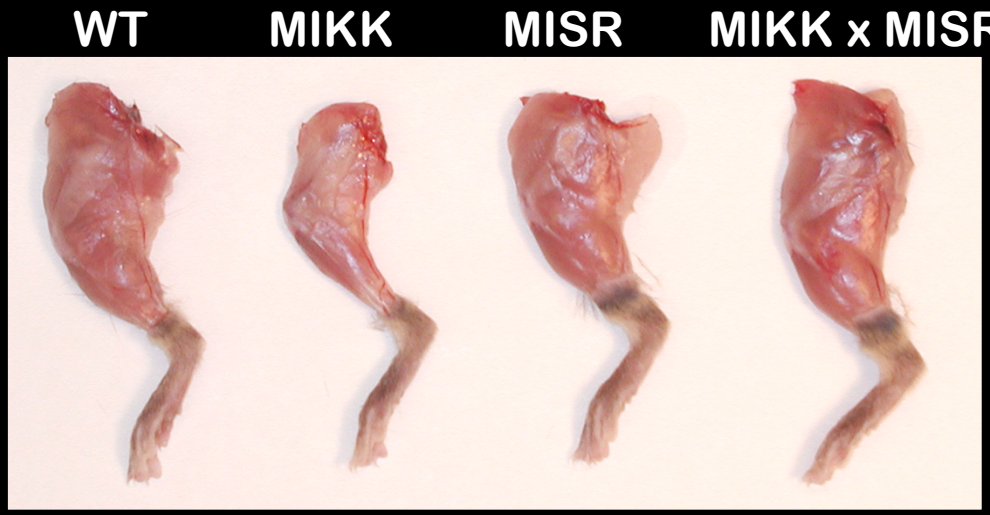


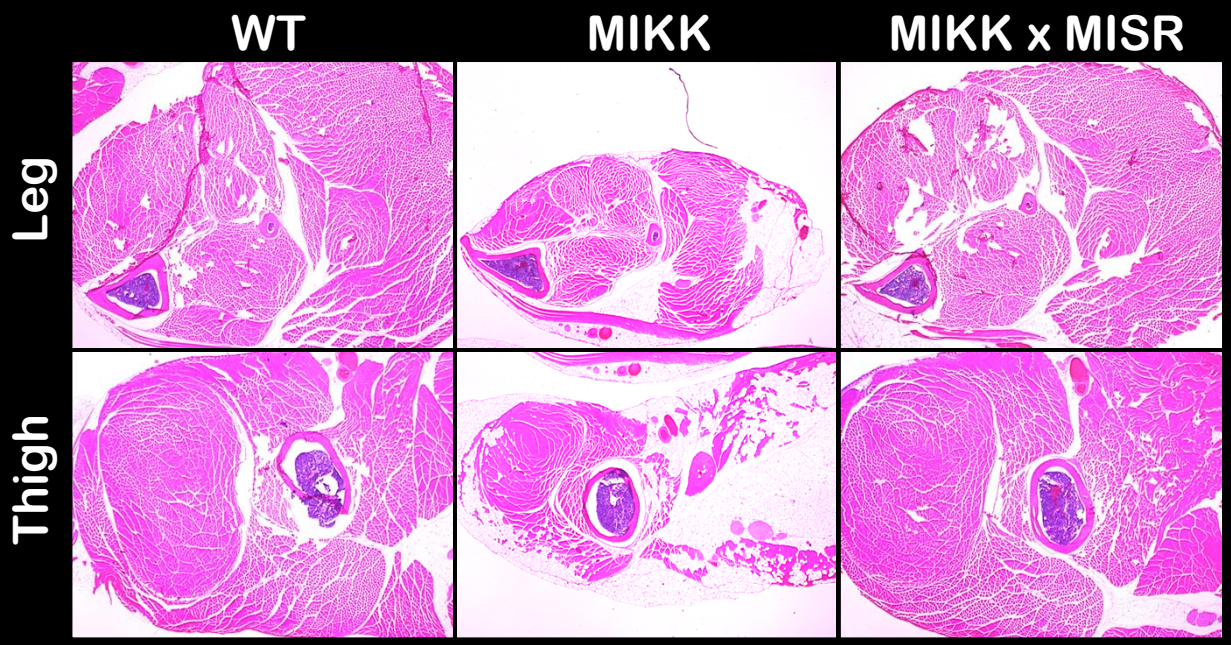






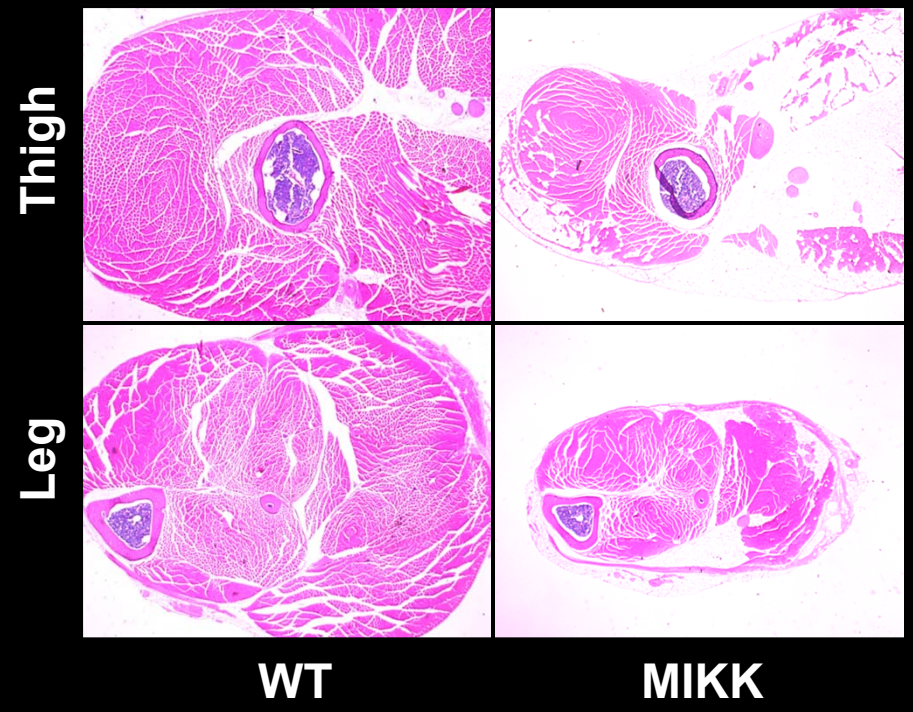
Cai…Shoelson, Cell *119*, 285-298 (2004).





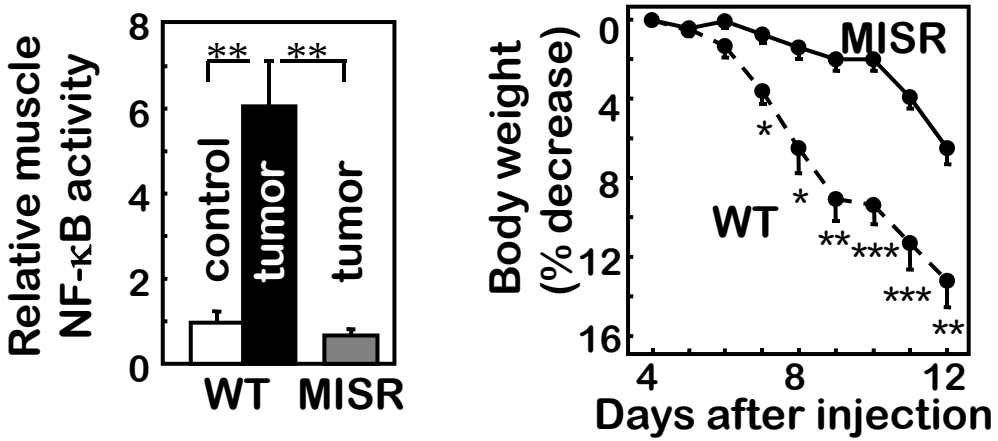
Cai…Shoelson, Cell *119*, 285-298 (2004).

Salicylate Reverses Muscle Wasting



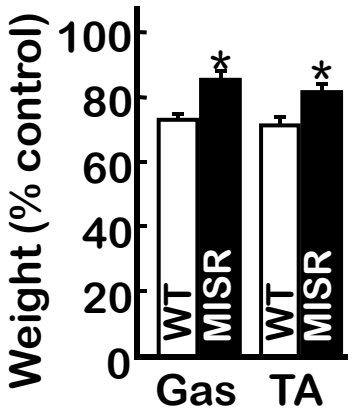
Cai et al., Cell *119*, 285 (2004)

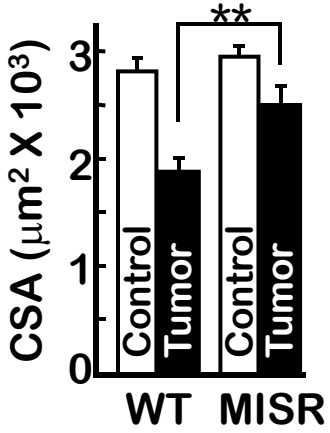
Tumor-induced muscle wasting

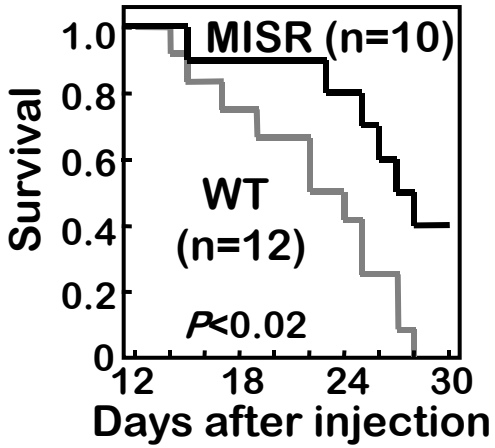


Cai…Shoelson, *Cell 119*, 285-298 (2004).

Cancer Cachexia





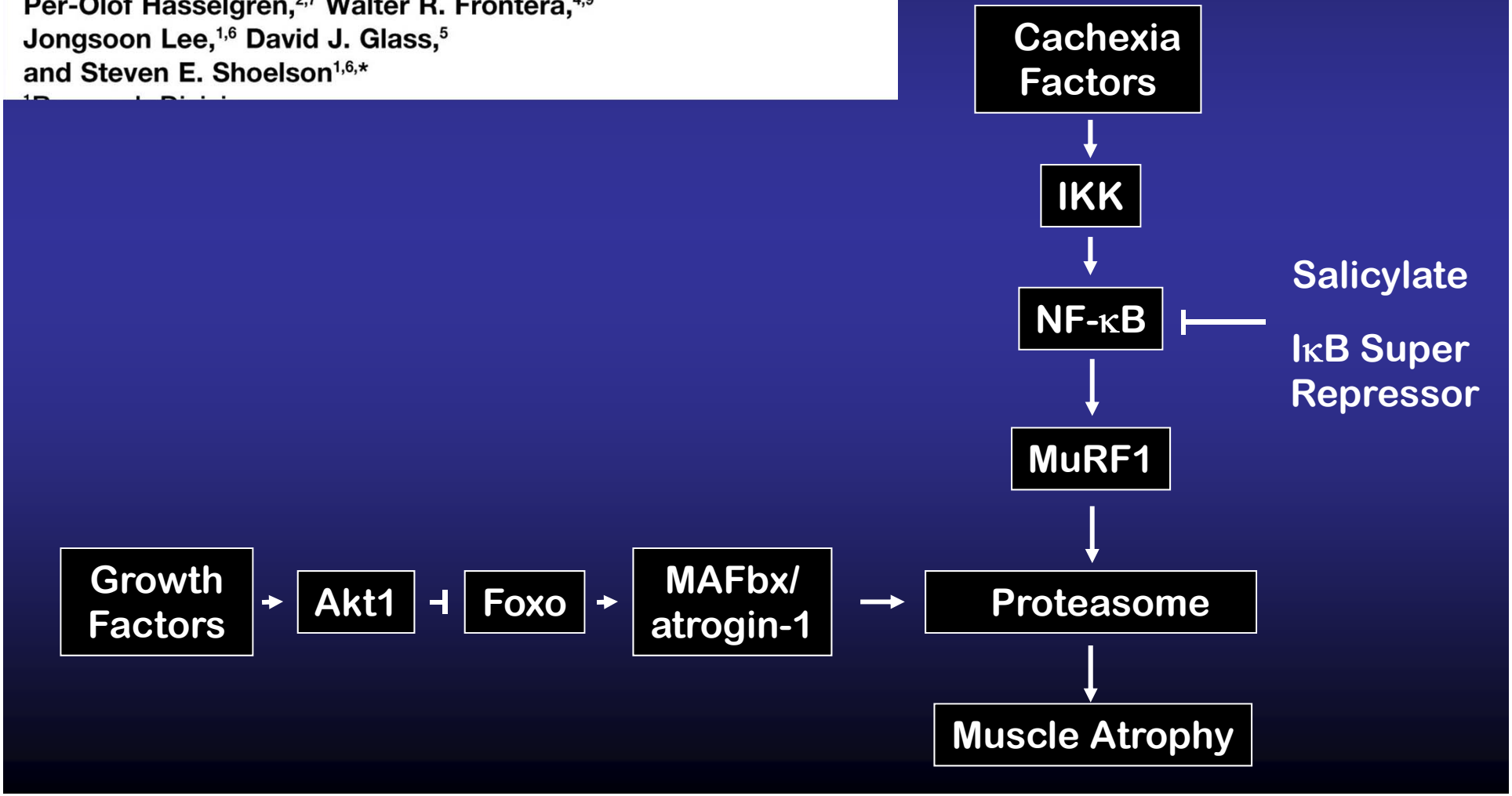


Cai…Shoelson, Cell *119*, 285-298 (2004).

Cell, Vol. 119, 285–298, October 15, 2004, Copyright ©2004 by Cell Press

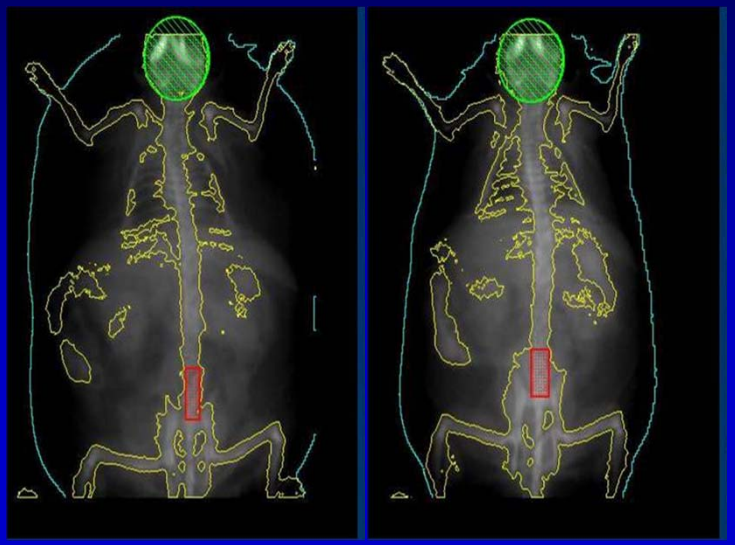
IKKβ/NF-κB Activation Causes Severe Muscle Wasting in Mice

Dongsheng Cai,[1,6] J. Daniel Frantz,[1,6,10]
Nicholas E. Tawa, Jr.,[2,7] Peter A. Melendez,[1,6,11]
Byung-Chul Oh,[1,6] Hart G.W. Lidov,[3,8]
Per-Olof Hasselgren,[2,7] Walter R. Frontera,[4,9]
Jongsoon Lee,[1,6] David J. Glass,[5]
and Steven E. Shoelson[1,6,*]

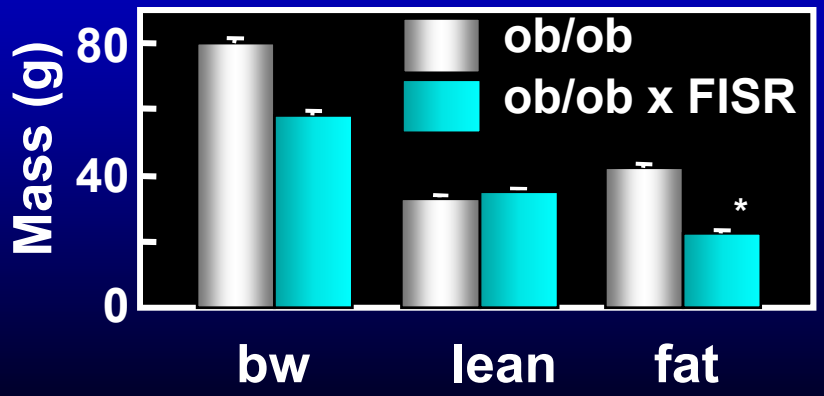


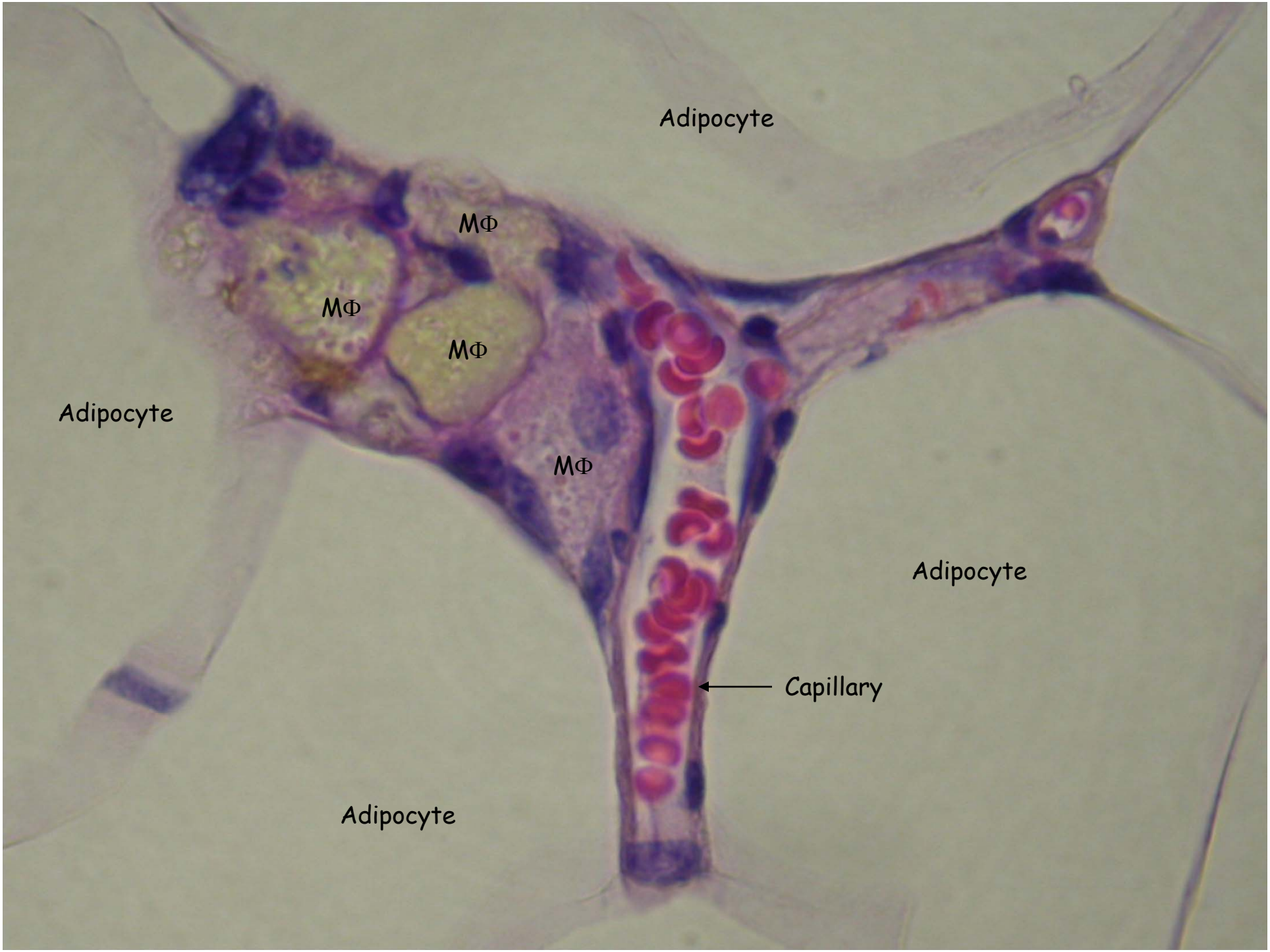
NF-kB inhibition in Adipocytes Protects Against Weight Gain

ob/ob ob/ob x FISR



ob/ob ob/ob x FISR




Adipocyte
MΦ
MΦ
MΦ
Adipocyte
MΦ
Adipocyte
Capillary
Adipocyte

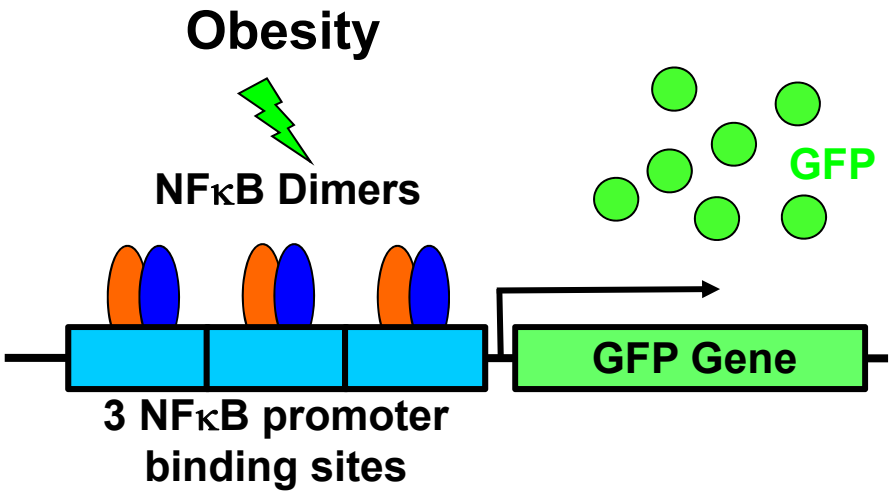
nature medicine

Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters

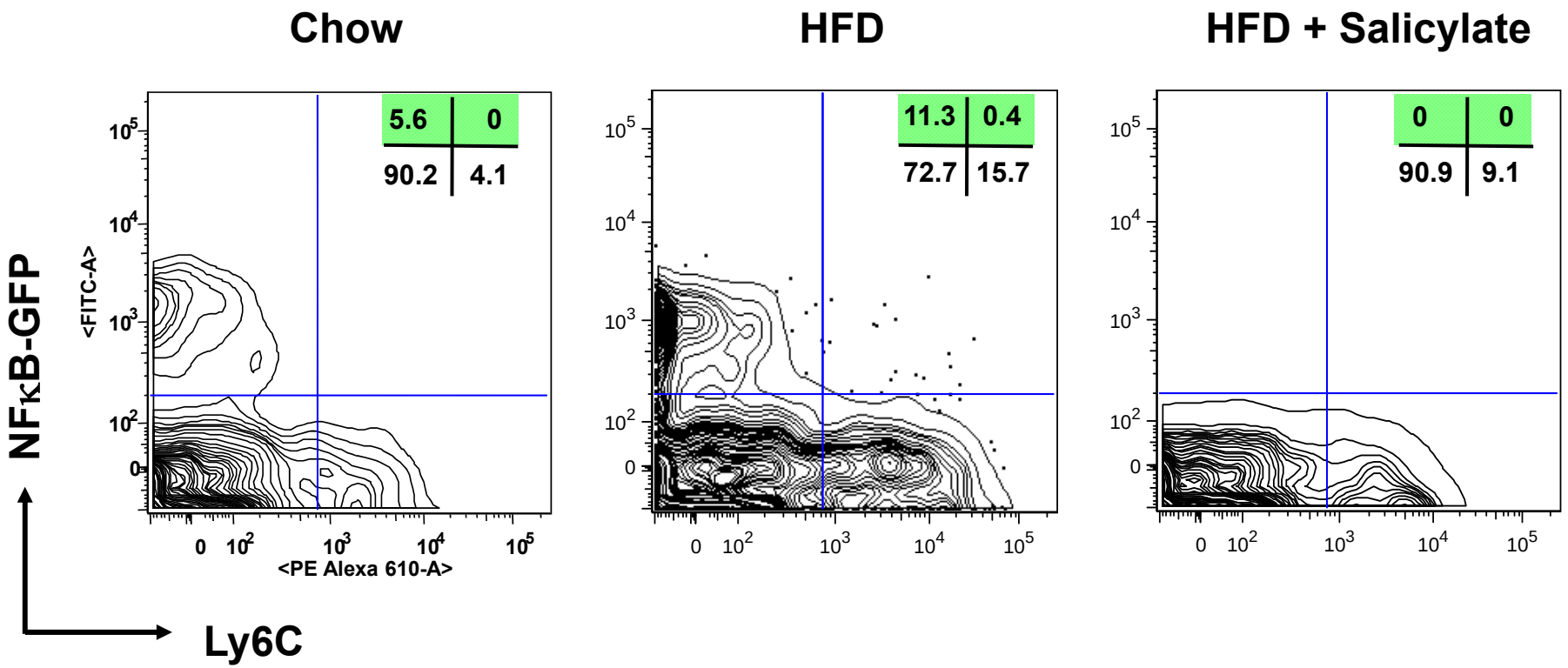
Markus Feuerer[1,5], Laura Herrero[2,5], Daniela Cipolletta[1,4,5], Afia Naaz[2], Jamie Wong[1,5], Ali Nayer[2], Jongsoon Lee[2], Allison B Goldfine[3], Christophe Benoist[1,5], Steven Shoelson[2] & Diane Mathis[1,5]

Obesity is accompanied by chronic, low-grade inflammation of adipose tissue, which promotes insulin resistance and type-2 diabetes. These findings raise the question of how fat inflammation can escape the powerful armamentarium of cells and molecules normally responsible for guarding against a runaway immune response. $CD4^+$ $Foxp3^+$ T regulatory (T_{reg}) cells with a unique phenotype were highly enriched in the abdominal fat of normal mice, but their numbers were strikingly and specifically reduced at this site in insulin-resistant models of obesity. Loss-of-function and gain-of-function experiments revealed that these T_{reg} cells influenced the inflammatory state of adipose tissue and, thus, insulin resistance. Cytokines differentially synthesized by fat-resident regulatory and conventional T cells directly affected the synthesis of inflammatory mediators and glucose uptake by cultured adipocytes. These observations suggest that harnessing the anti-inflammatory properties of T_{reg} cells to inhibit elements of the metabolic syndrome may have therapeutic potential.

NFκB-GFP Transgenic Mice

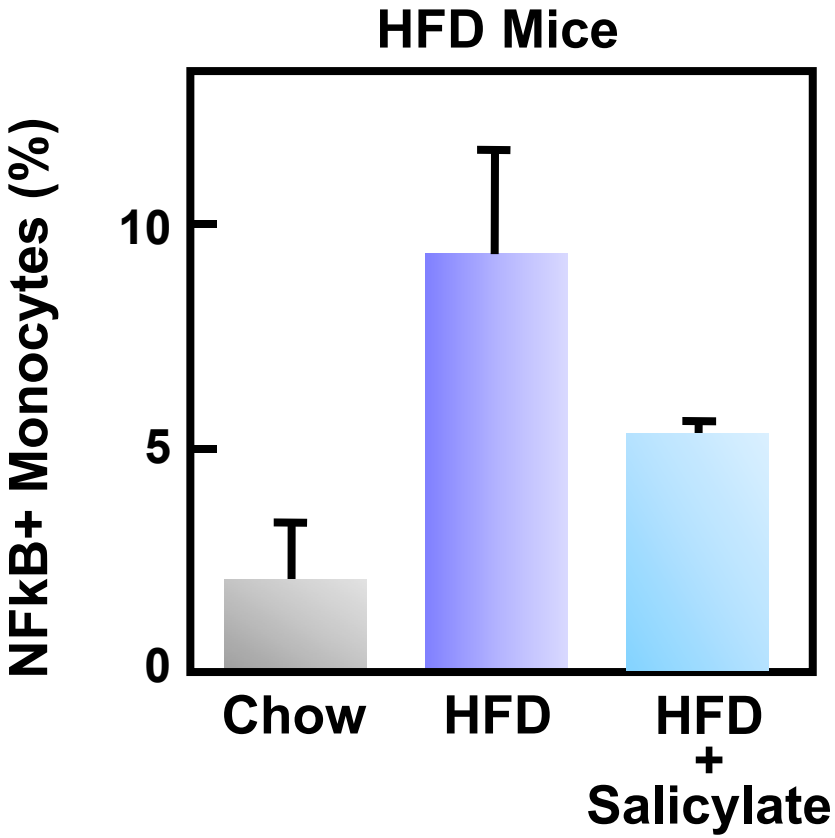


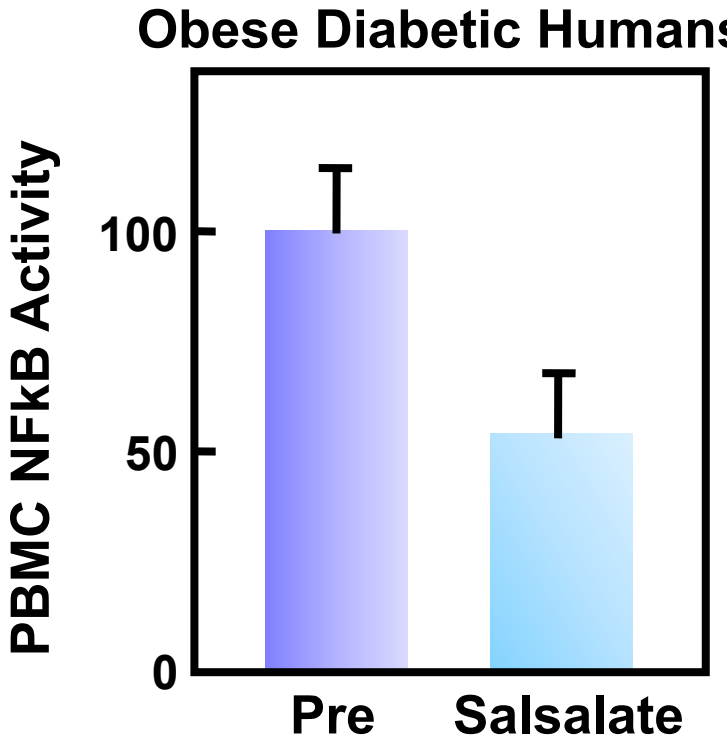
Obesity Activates NFκB+ and Salicylate Inhibits NFκB+ in Circulating Monocytes



Yasuhiko Yamamoto & Jongsoon Lee

Obesity Activates NF-κB and Salicylate Inhibits NF-κB in Circulating Monocytes





15 week HFD / 4 week Salicylate

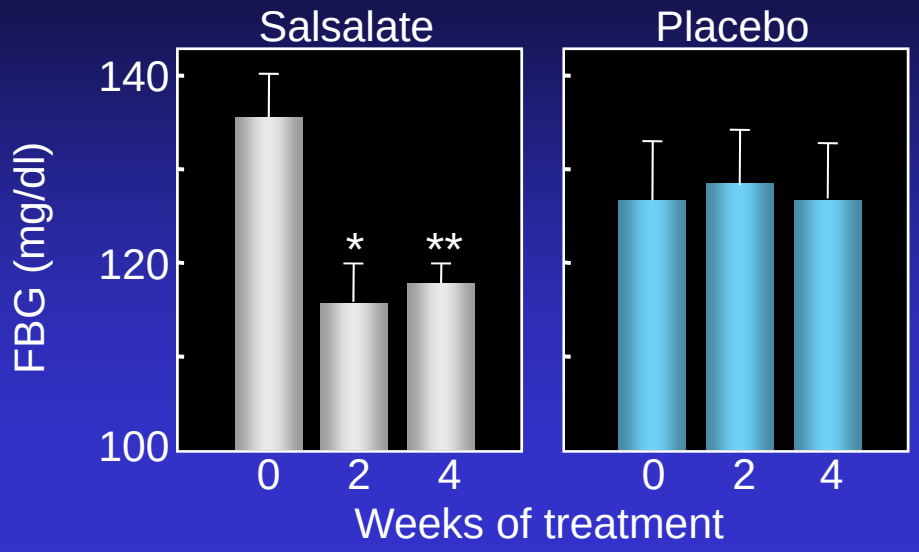
Goldfine…Shoelson, CTS, 2008;1;36

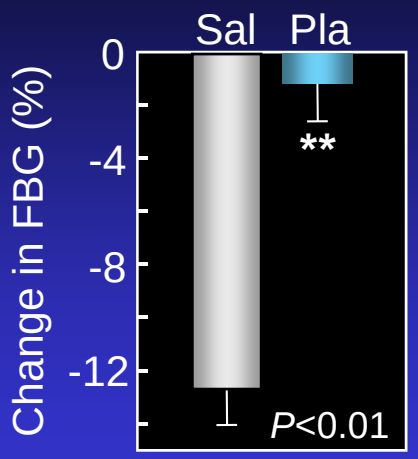
Human Trials?

Is inflammation a practical therapeutic target in type 2 diabetes?

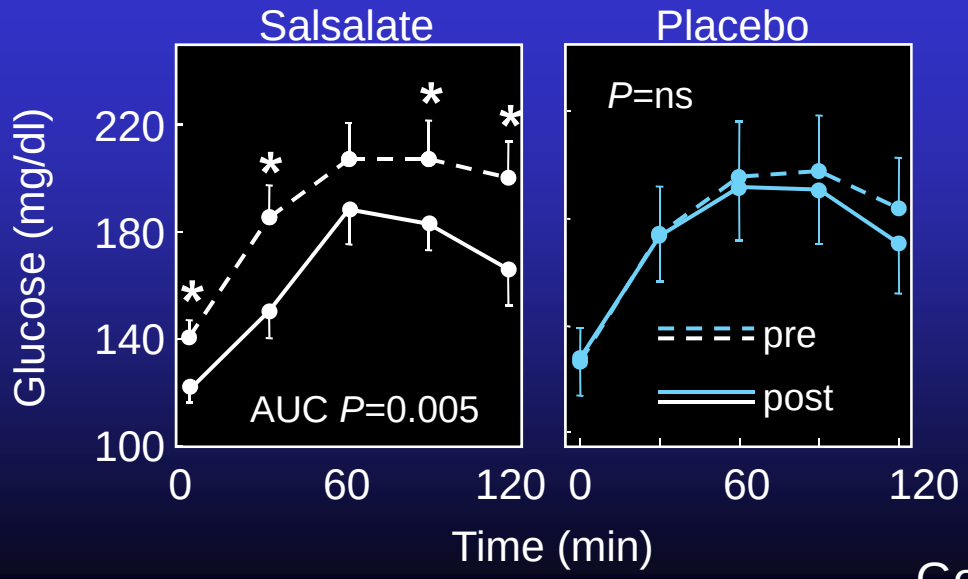
Do anti-inflammatory strategies prevent diabetes and decrease risk of CVD?

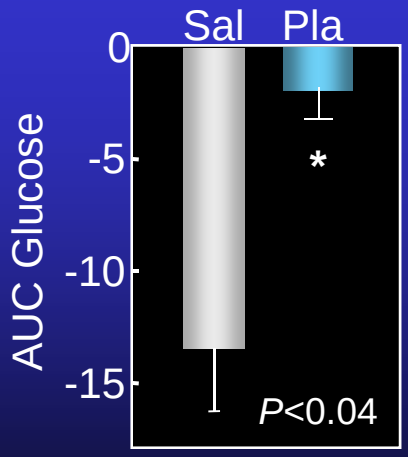
4.0 g/d salsalate in T2D: 4 weeks duration/placebo controlled





Mixed meal tolerance test





Goldfine…Shoelson, CTS, 2008;1;36

TINSAL-T2D Stage 1

Targeting Inflammation with Salsalate in Type 2 Diabetes, a NIDDK-Sponsored Trial

Steve Shoelson, PI Allison Goldfine, Co-PI Vivian Fonseca, Co-PI

Kathleen Jablonski, DCC/GWU Myrlene Staten, NIDDK

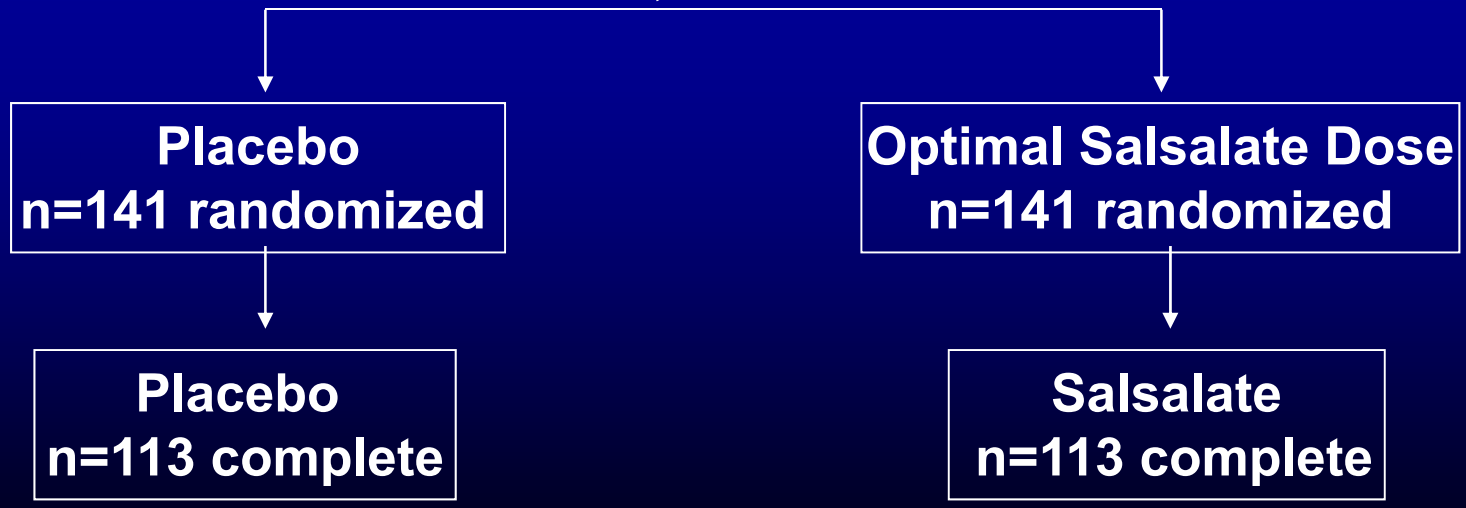
TINSAL-T2D Trial Design

Stage I: 14-week multicenter, double-masked, placebo-controlled dose ranging study in inadequately controlled T2D ($7.0 < HbA1c \leq 9.5$)

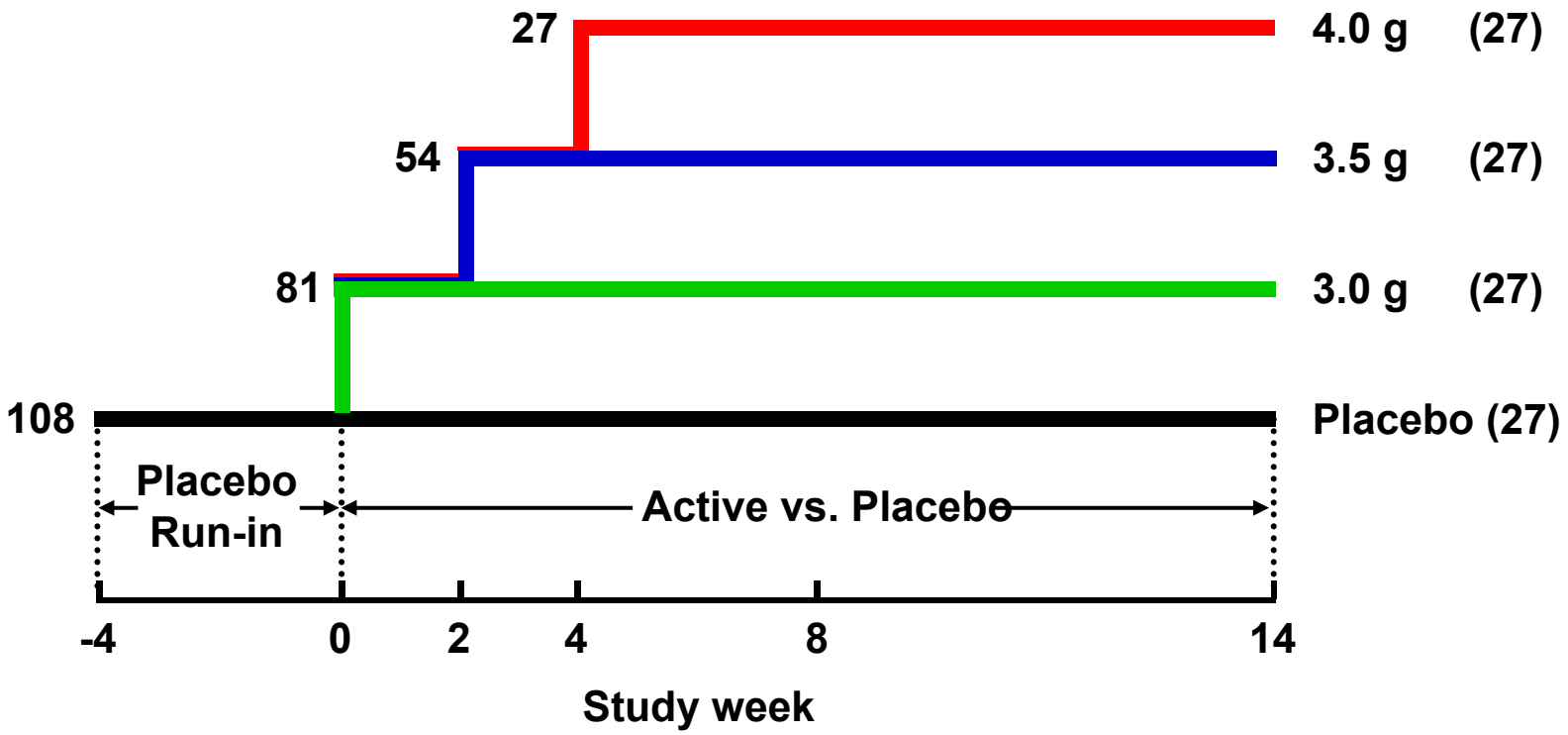
Screen n=240, Randomize n=108

Stage II: 26-week multicenter, double-masked, placebo-controlled phase III trial

Screen n=564, Randomize n=282



TINSAL-T2D* Stage 1 Trial Design



*TINSAL-T2D is an NIH/NIDDK sponsored multi-center, randomized, double-masked, placebo-controlled, dose ranging parallel-group clinical trial.

TINSAL-T2D Stage 1: Baseline Patient Characteristics 1

	Treatment Arm (n)				
	Placebo (27)	3.0 g/d (27)	3.5 g/d (27)	4.0 g/d (27)	P value
Age (yr)	55.9 ± 8.2	55.4 ± 9.4	56.7 ± 9.8	55.0 ± 10.2	0.92
Male / Female (%)	56 / 44	52 / 48	67 / 33	59 / 41	0.72
White / Black / Other (%)	56 / 33 / 11	44 / 46 / 7	52 / 37 / 11	56 / 40 / 4	0.94
Weight (kg)	99.1 ± 22.1	92.9 ± 22.2	97.7 ± 18.8	102 ± 20.7	0.43
BMI (kg/m^2)	34.0 ± 6.1	32.3 ± 6.8	32.9 ± 6.5	35.0 ± 6.5	0.42
Waist circumference (cm)	104 ± 22.7	103 ± 15.6	106 ± 18.7	109 ± 23.0	0.79
Years since T2D diagnosed	5.1 ± 3.7	6.4 ± 5.2	6.9 ± 6.0	6.4 ± 4.4	0.58

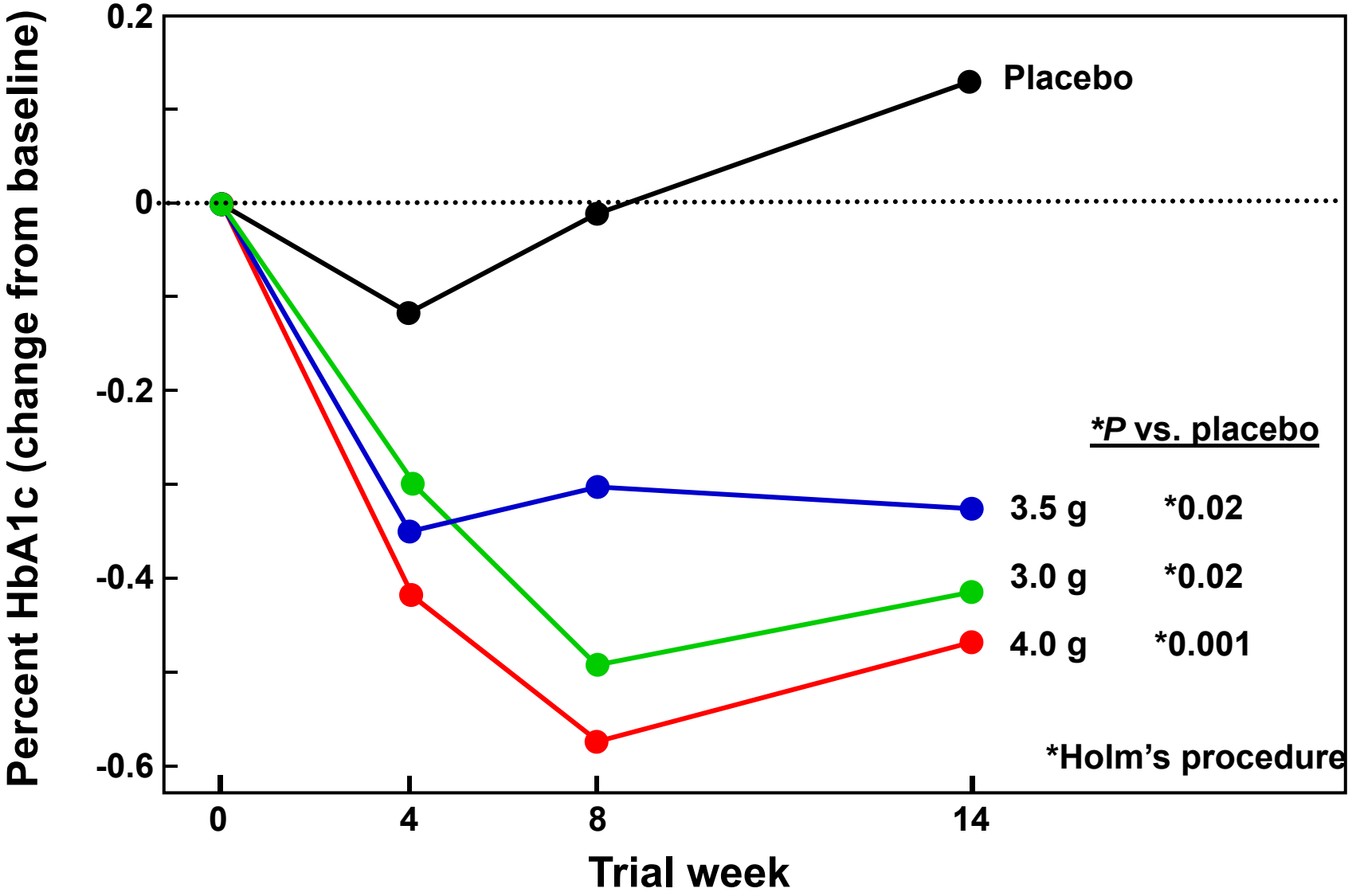
TINSAL-T2D Stage 1: Baseline Patient Characteristics 2

	Treatment Arm (n)				
	Placebo (27)	3.0 g/d (27)	3.5 g/d (27)	4.0 g/d (27)	P value
Physical Findings					
Systolic BP (mm Hg)	125 ± 14.4	125 ± 12.8	124 ± 13.2	128 ± 11.6	0.67
Diastolic BP (mm Hg)	76 ± 8.2	79 ± 7.4	77 ± 9.5	76 ± 9.3	0.72
Heart rate (bpm)	74.0 ± 10.9	72.4 ± 8.6	73.5 ± 13.2	71.8 ± 7.1	0.85
Laboratory Values					
Cholesterol (mg/dl)	180 ± 48.6	164 ± 50.0	186 ± 54.4	170 ± 26.8	0.32
Triglycerides (mg/dl)	183 ± 92.8	184 ± 178	151 ± 75.6	160 ± 80.7	0.66
Fasting glucose (mg/dl)	153 ± 39.8	154 ± 41.5	149 ± 38.5	144 ± 36.2	0.76
HbA1c (%)	7.8 ± 0.8	7.9 ± 1.1	7.4 ± 0.7	7.6 ± 0.9	0.25

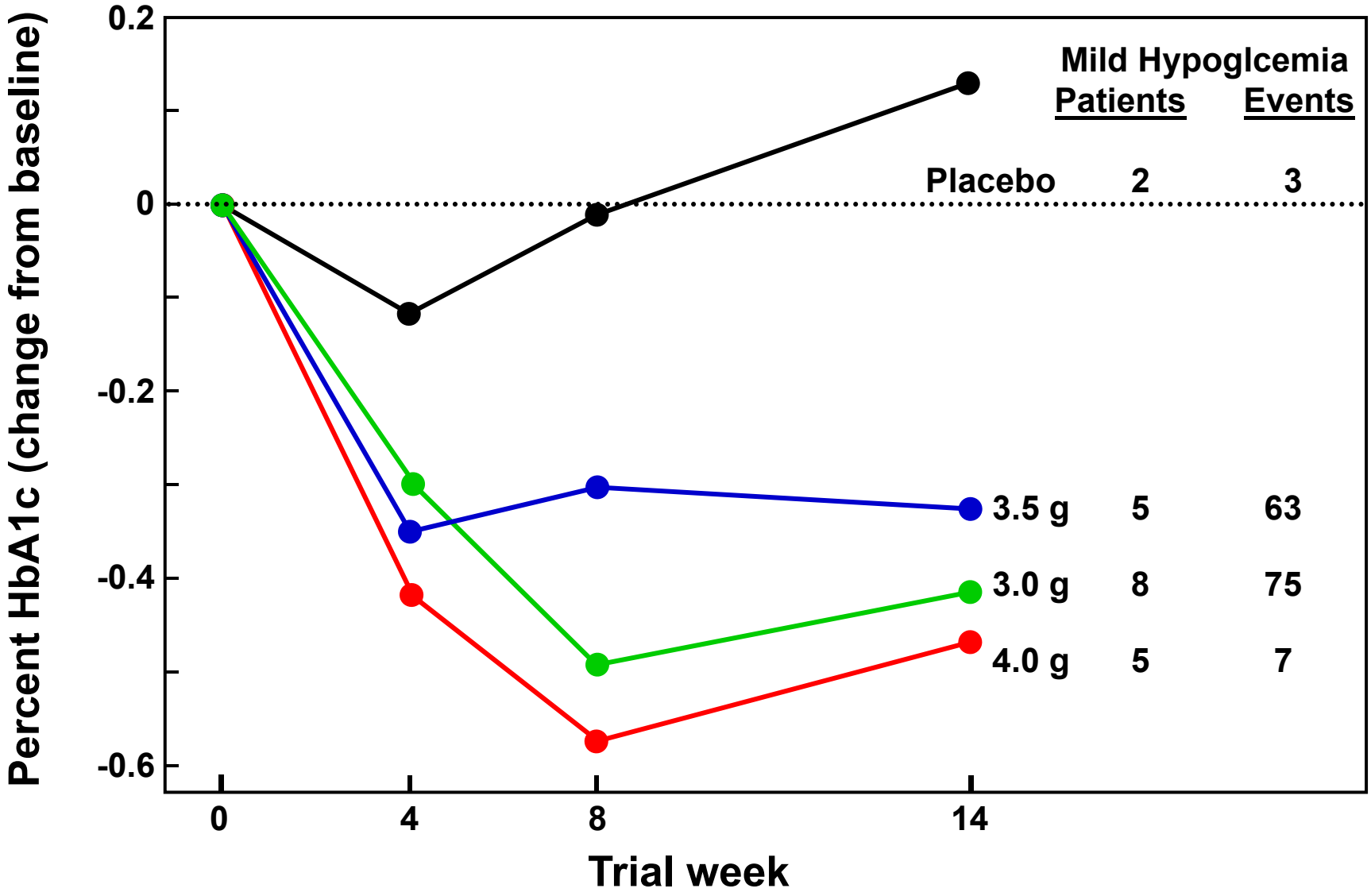
TINSAL-T2D Stage 1: Baseline Patient Characteristics 3

	Treatment Arm (n)				
	Placebo (27)	3.0 g/d (27)	3.5 g/d (27)	4.0 g/d (27)	P value
Ongoing diabetes therapies (%)					
Lifestyle only	14.8	14.8	18.5	11.1	0.98
Metformin	81.5	81.5	66.7	85.6	0.35
Insulin secretagogue	48.1	37.0	44.4	44.4	0.94
α-Glucosidase inhibitor	0	0	0	0	
DPP-4 inhibitor	3.7	3.7	0	0	
Two or more DM drugs	37.4	28.7	17.9	34.7	0.76

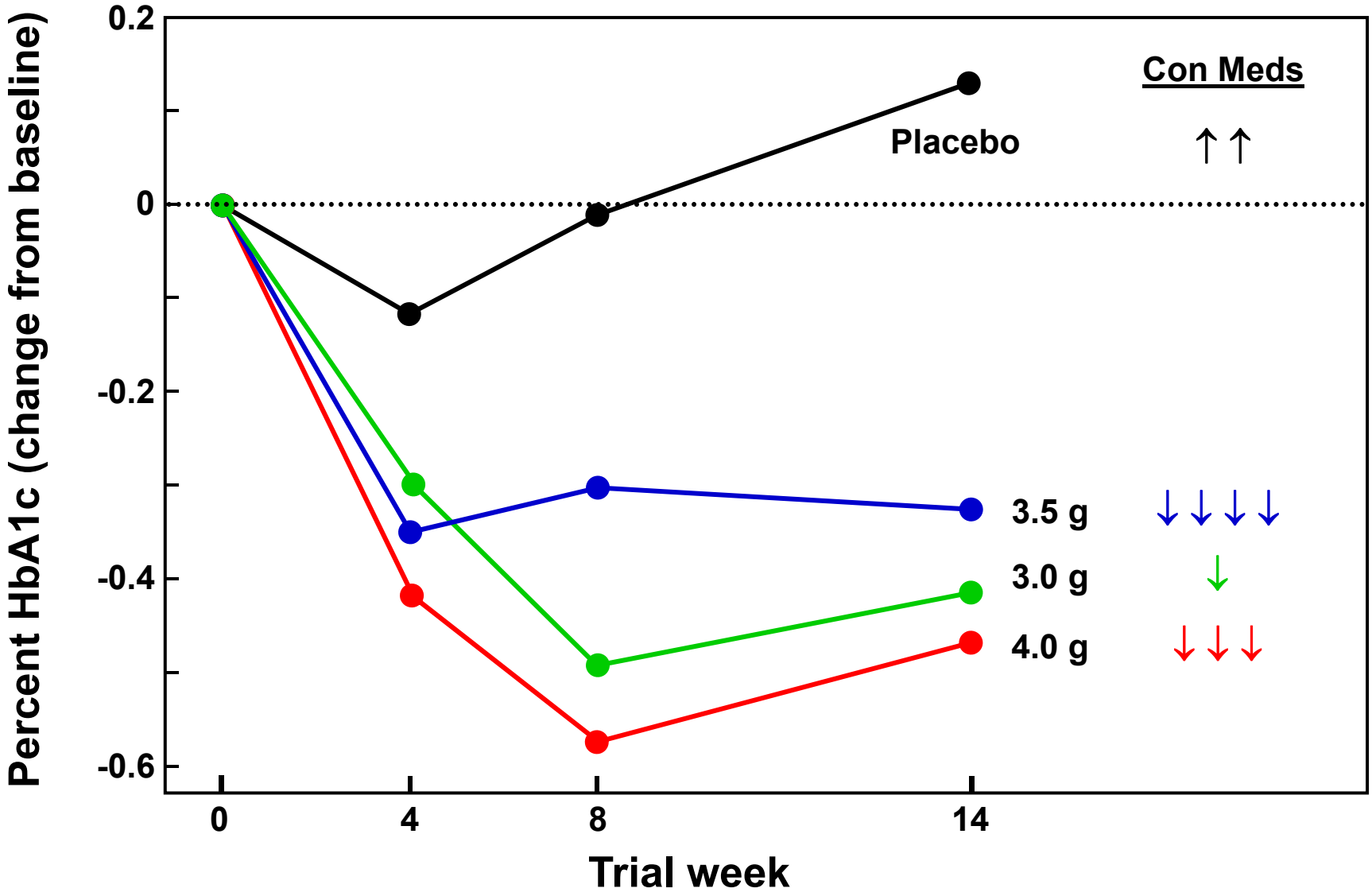
TINSAL-T2D Stage 1: HbA1c (primary endpoint)



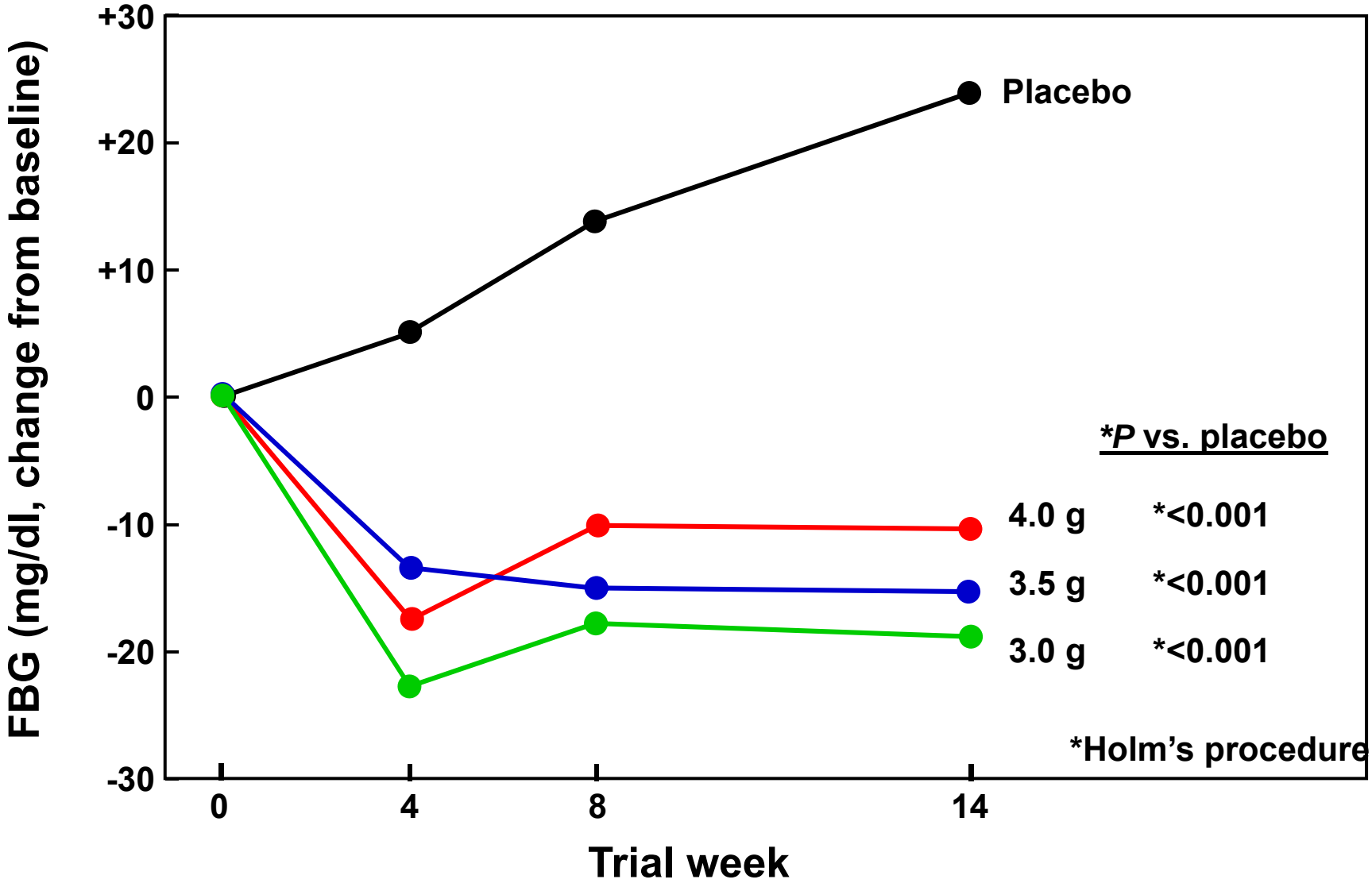
TINSAL-T2D Stage 1: HbA1c (primary endpoint)



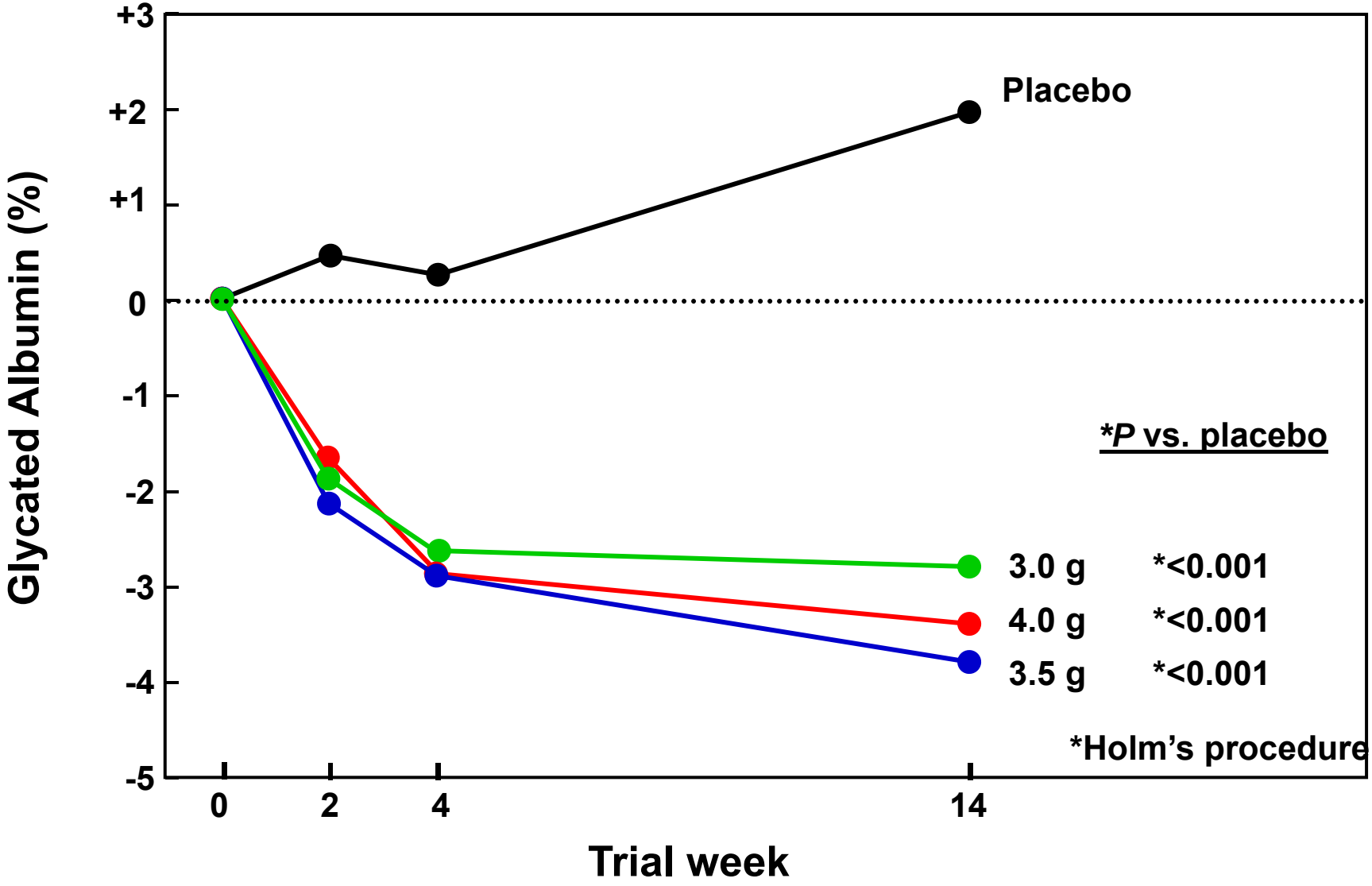
TINSAL-T2D Stage 1: HbA1c (primary endpoint)



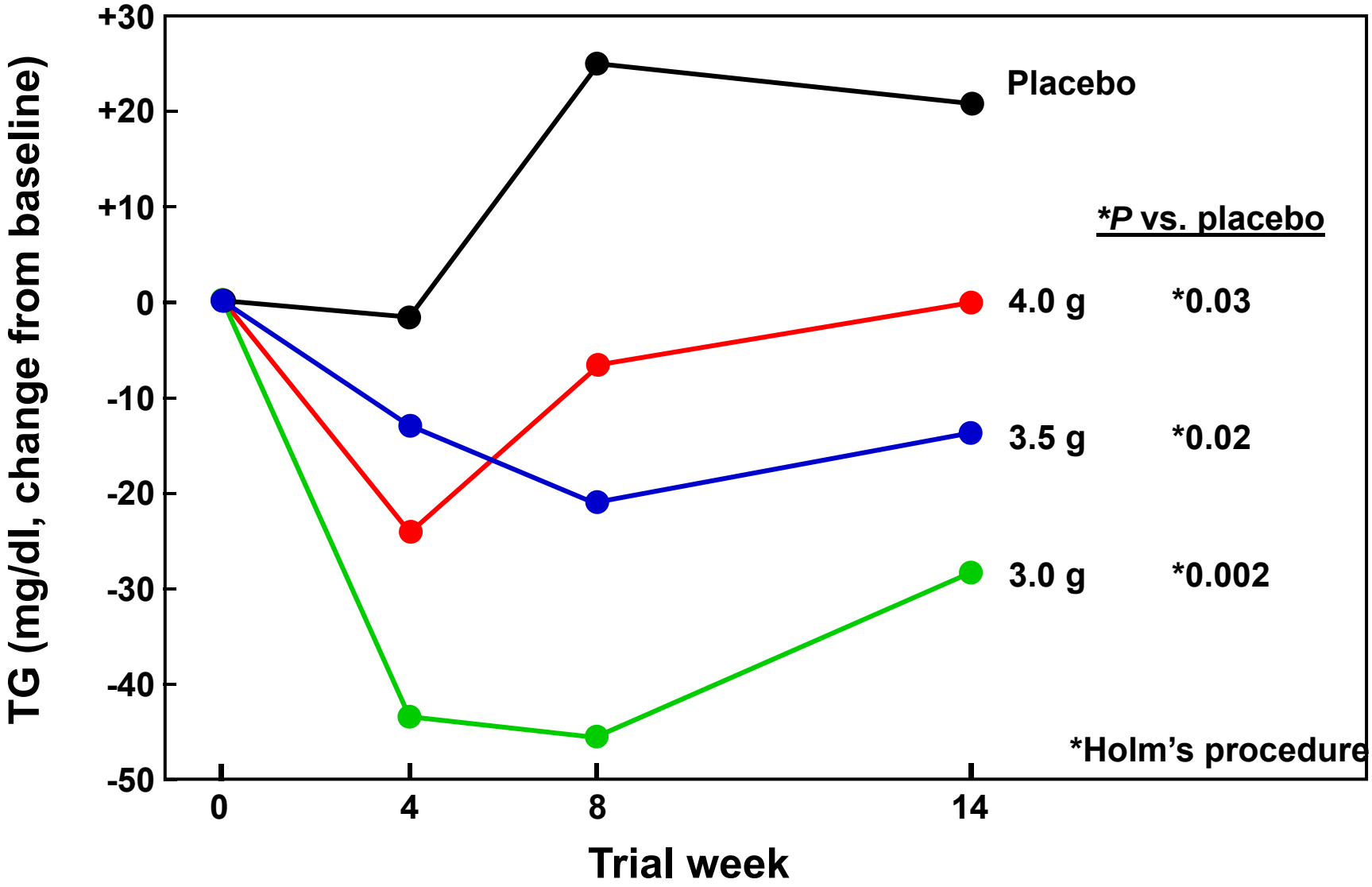
TINSAL-T2D Stage 1: Fasting Blood Glucose



TINSAL-T2D Stage 1: Fasting Blood Glucose

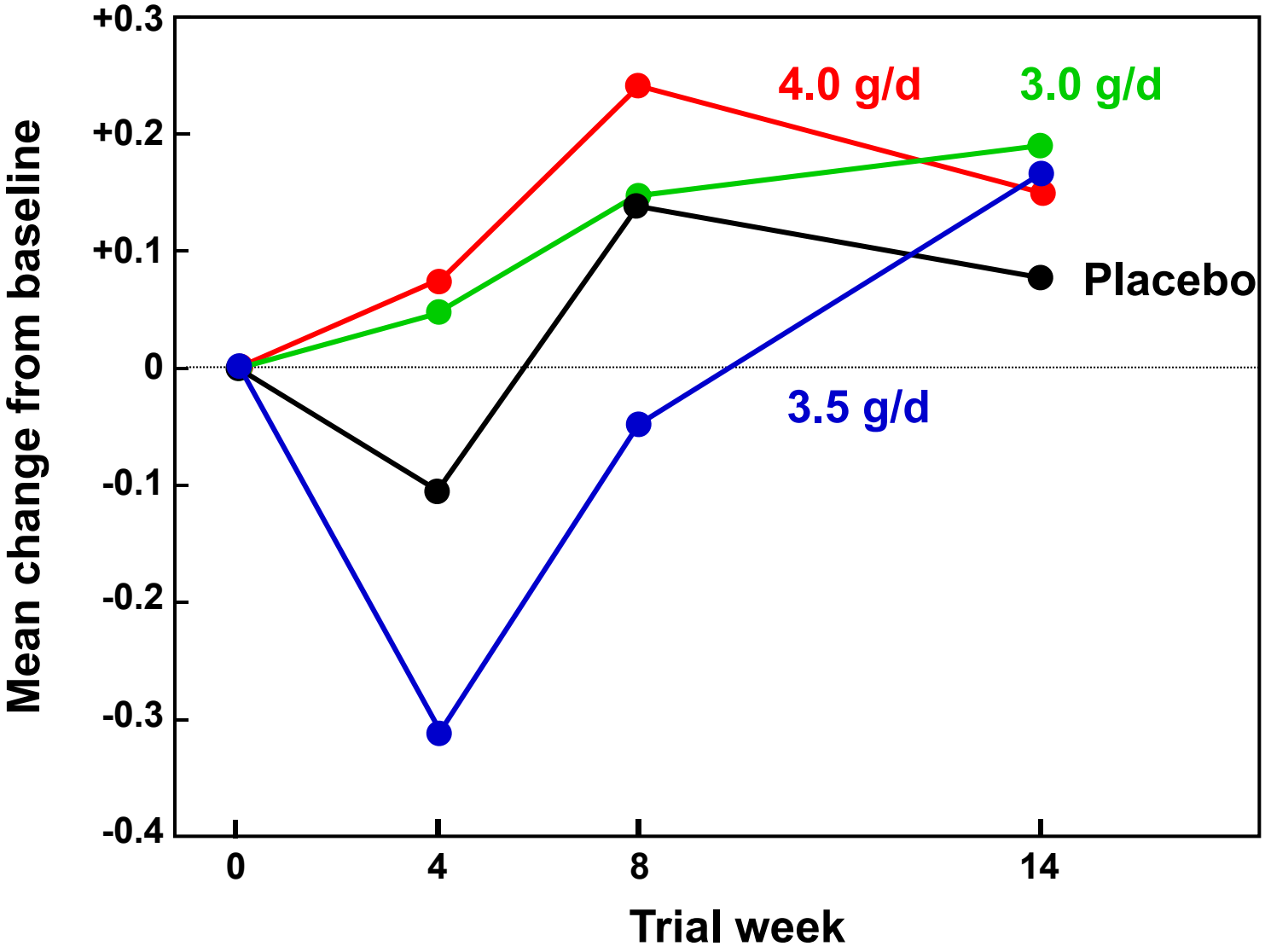


TINSAL-T2D Stage 1: Triglycerides



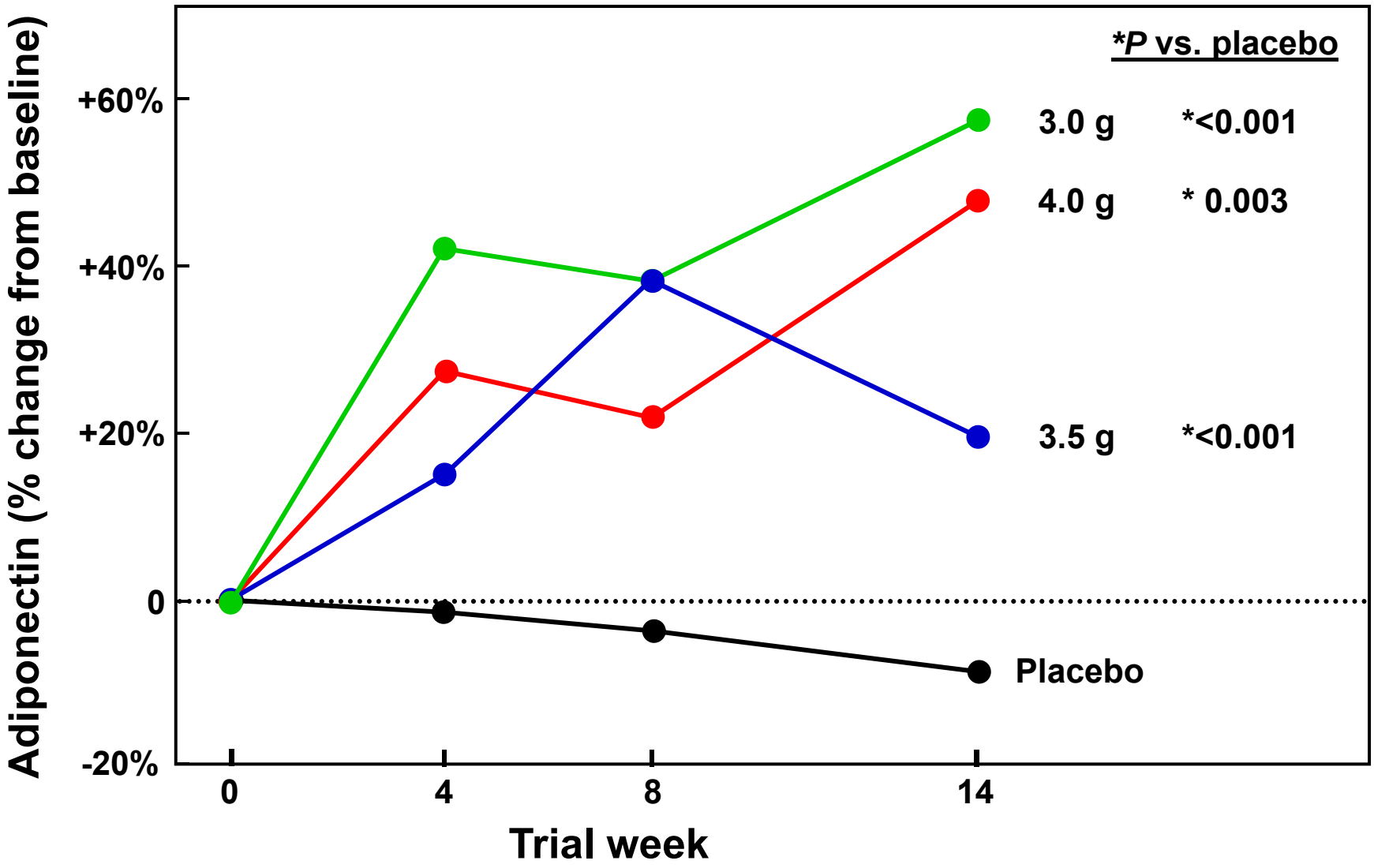
The F-test for the overall effect of treatment group was significant (p=0.005)

TINSAL-T2D Stage 1: Cholesterol, Total / HDL



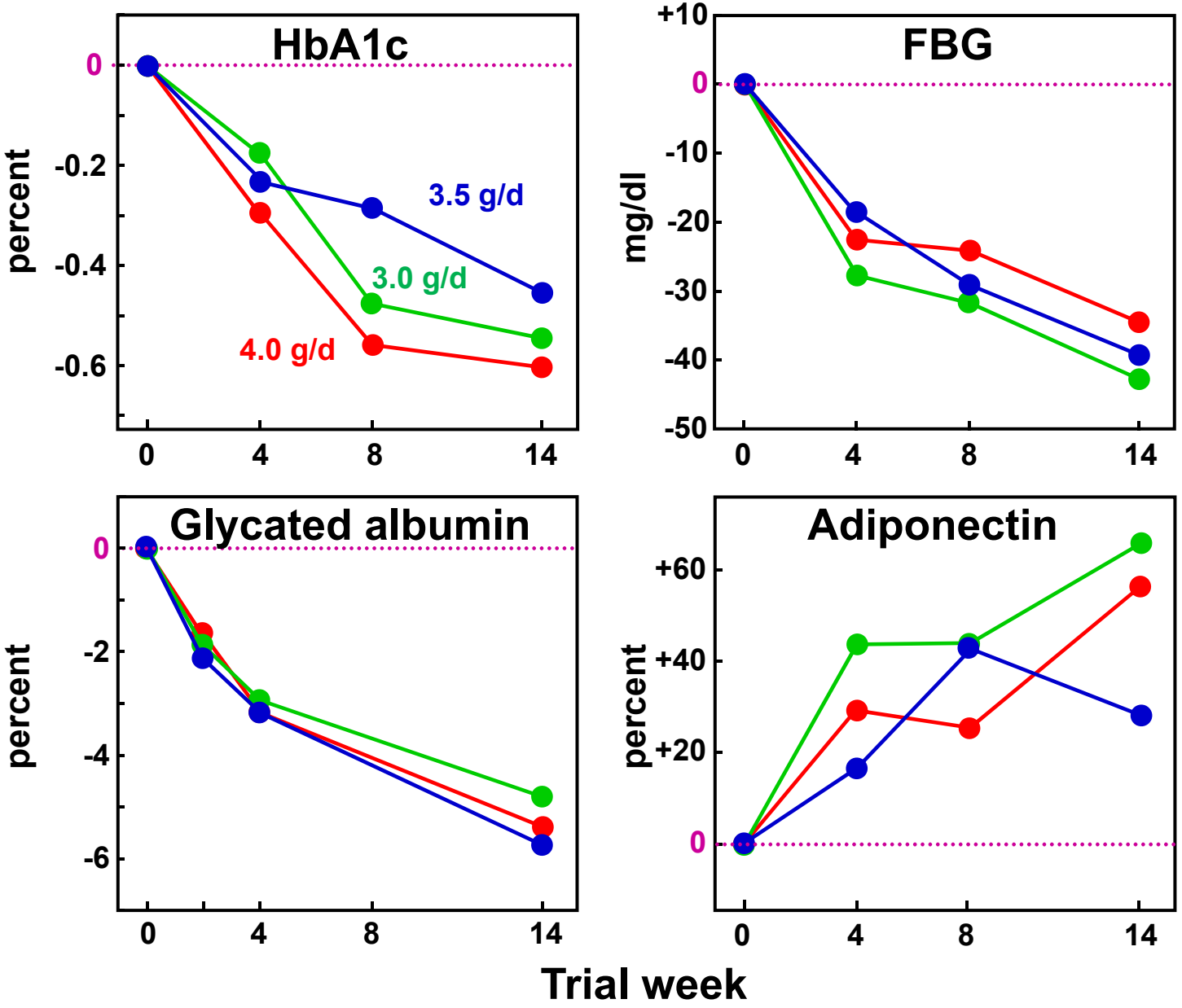
The F-test for the overall effect of treatment was not significant (p=0.5542).

TINSAL-T2D Stage 1: Adiponectin



*T-test adjusted for multiple comparisons using Holm's procedure

TINSAL-T2D, Placebo Adjusted Change form Baseline



Other pertinent negatives

- No change in body weight
- No change in LFTs (AST, ALT, GGT, etc.)
- No change in electrolytes or anion gap
- No change in thyroid function (TSH)
- No significant GI toxicity or evidence of GI bleeding

Other pertinent positives

- Salsalate was well tolerated, with high compliance and low rates of dropout
- Lower than expected rates of tinnitus
- Subjects reported improvements in sense of health and well being (SF36), including improvements in both physical and social functioning

TINSAL-T2D Trial Design

Stage I: 14-week multicenter, double-masked, placebo-controlled dose ranging study in inadequately controlled T2D (7.0 ≤ HbA1c ☐ 9.5)

Screen n=240, Randomize n=108

- Placebo n=27
- 3.0g n=27
- 3.5g n=27
- 4.0g n=27

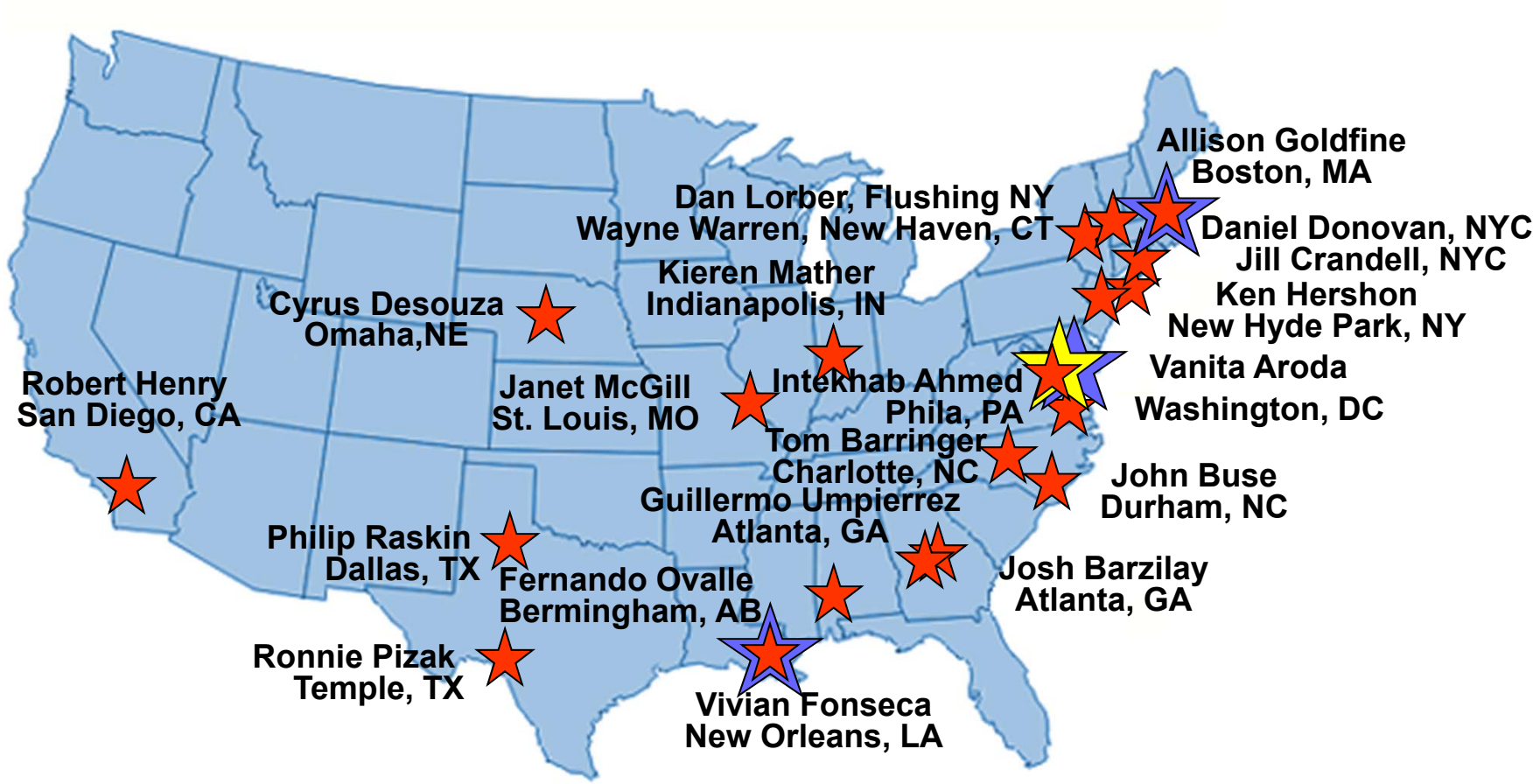
Stage II: 52-week multicenter, double-masked, placebo-controlled phase III trial

Screen n=564, Randomize n=282

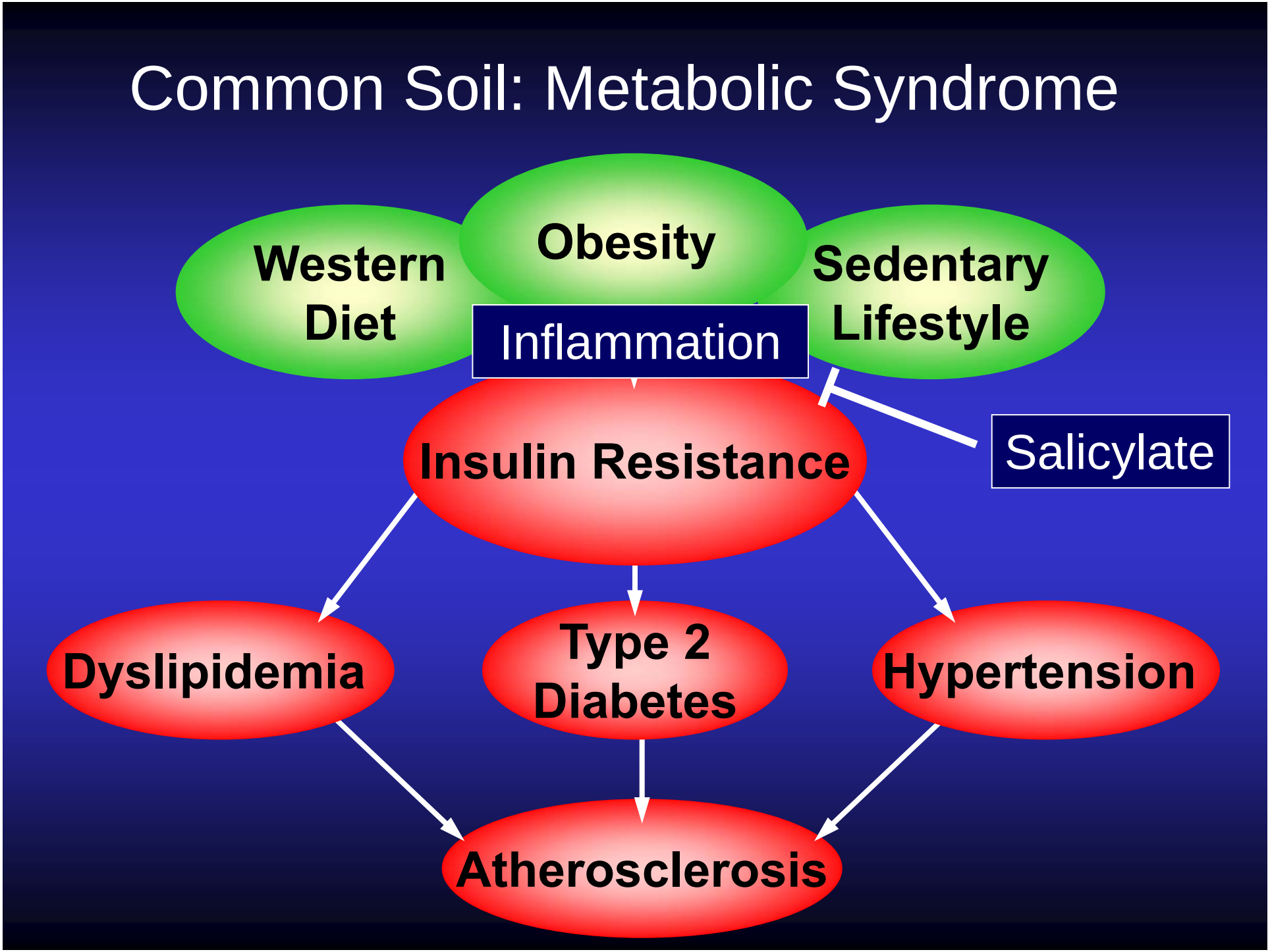
- Placebo n=141 randomized
  - Placebo n=113 complete
- Salsalate 3.5 g/d n=141 randomized
  - Salsalate n=113 complete

TINSAL-T2D Stage 2

An NIDDK funded clinical trial

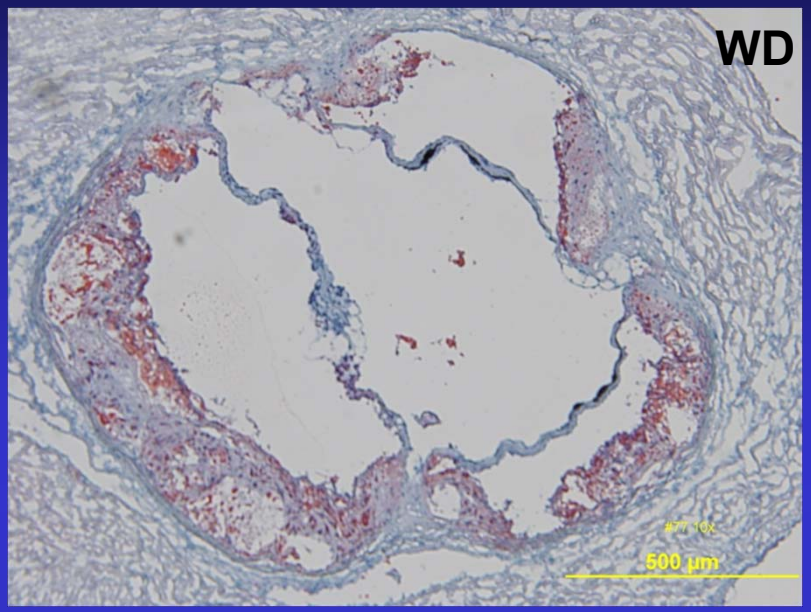


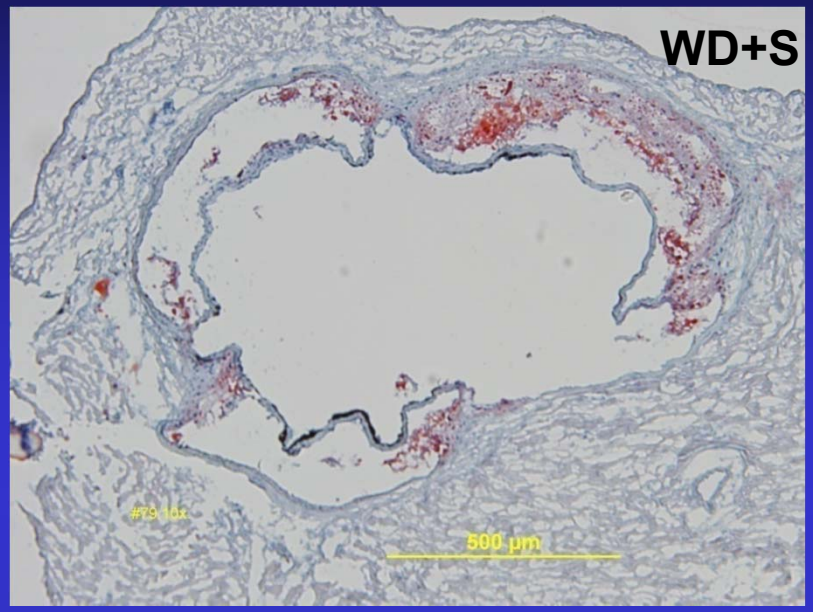
Common Soil: Metabolic Syndrome

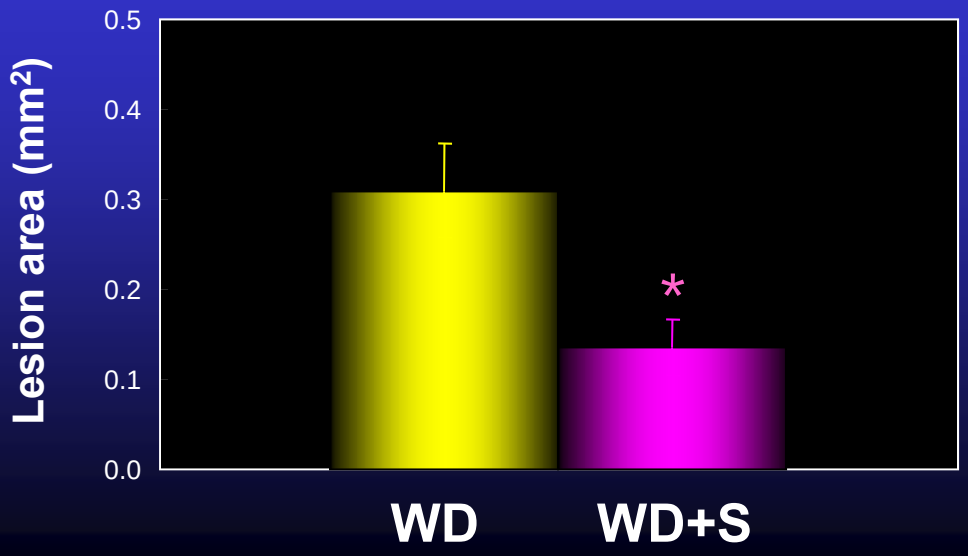


Quantitation of Atherosclerotic Lesions

(Aortic Root, 16 weeks on WD±S)



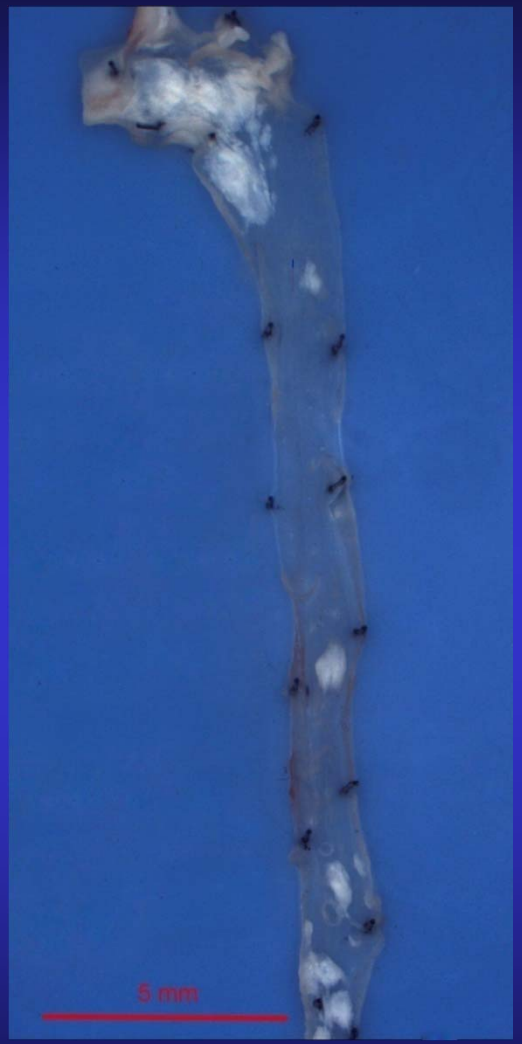




Tatjana Ignjatovic

Ldlr-/- mice on diet for 6 months

Chow



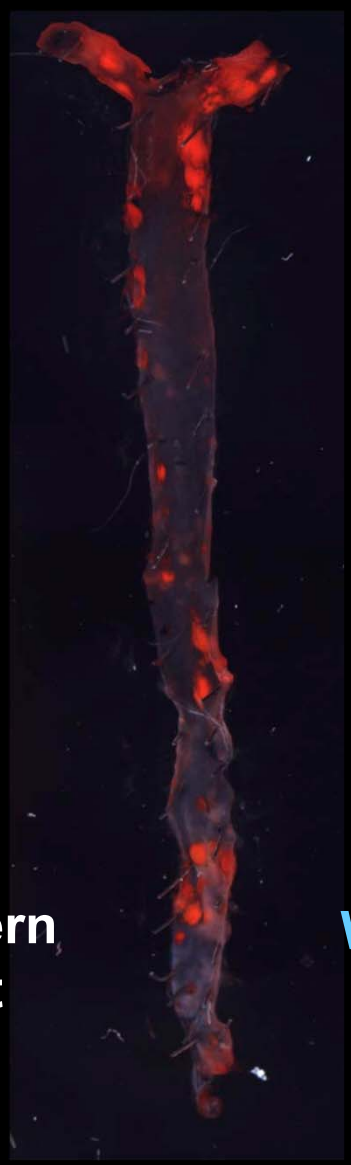
Western Diet

Western Diet + Salicylate

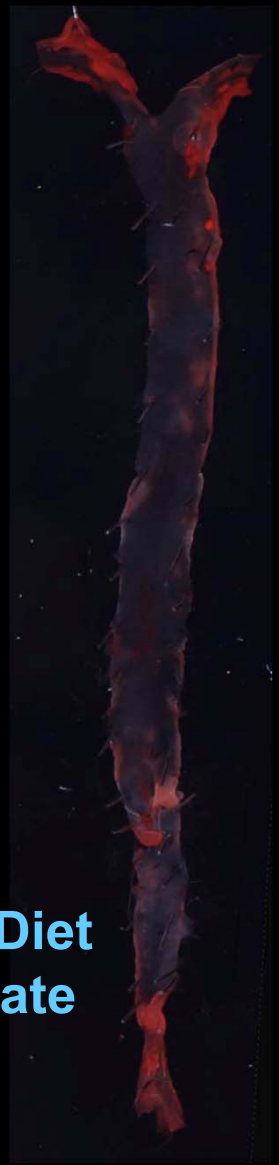
Ignjatovic and Shoelson, unpublished

Quantitation of Atherosclerotic Lesions

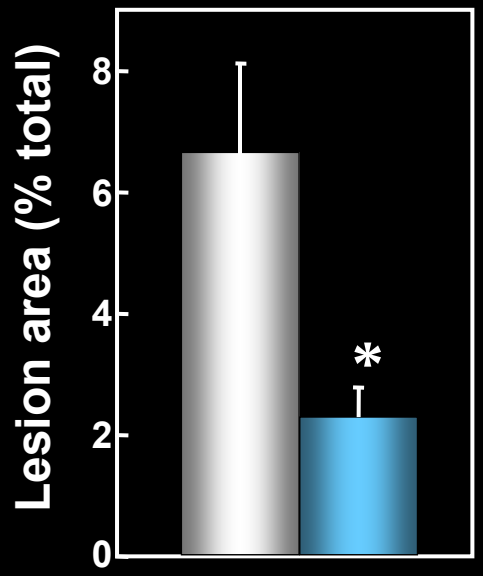
(En face, 16 weeks on WD±S)



Western Diet

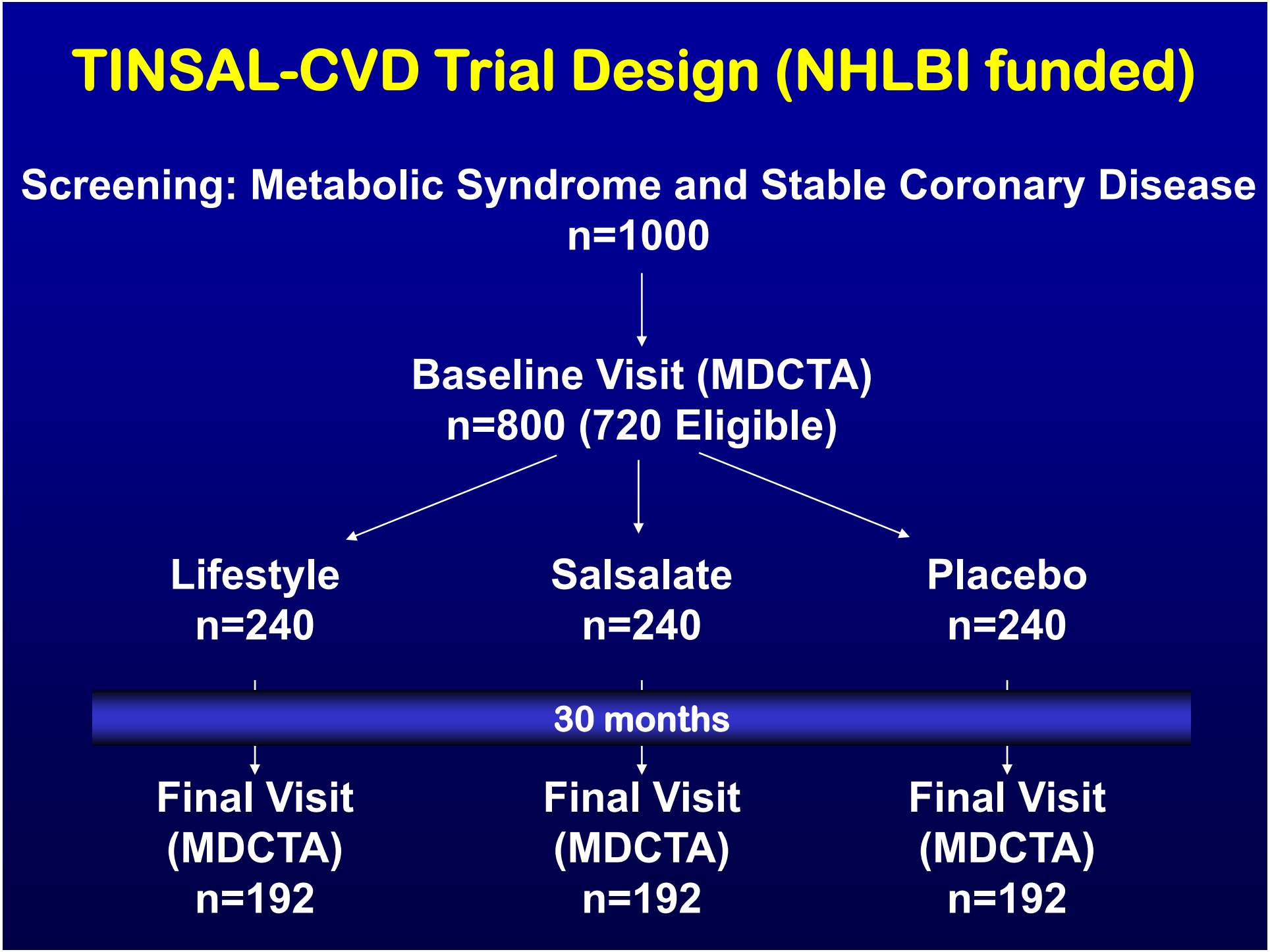


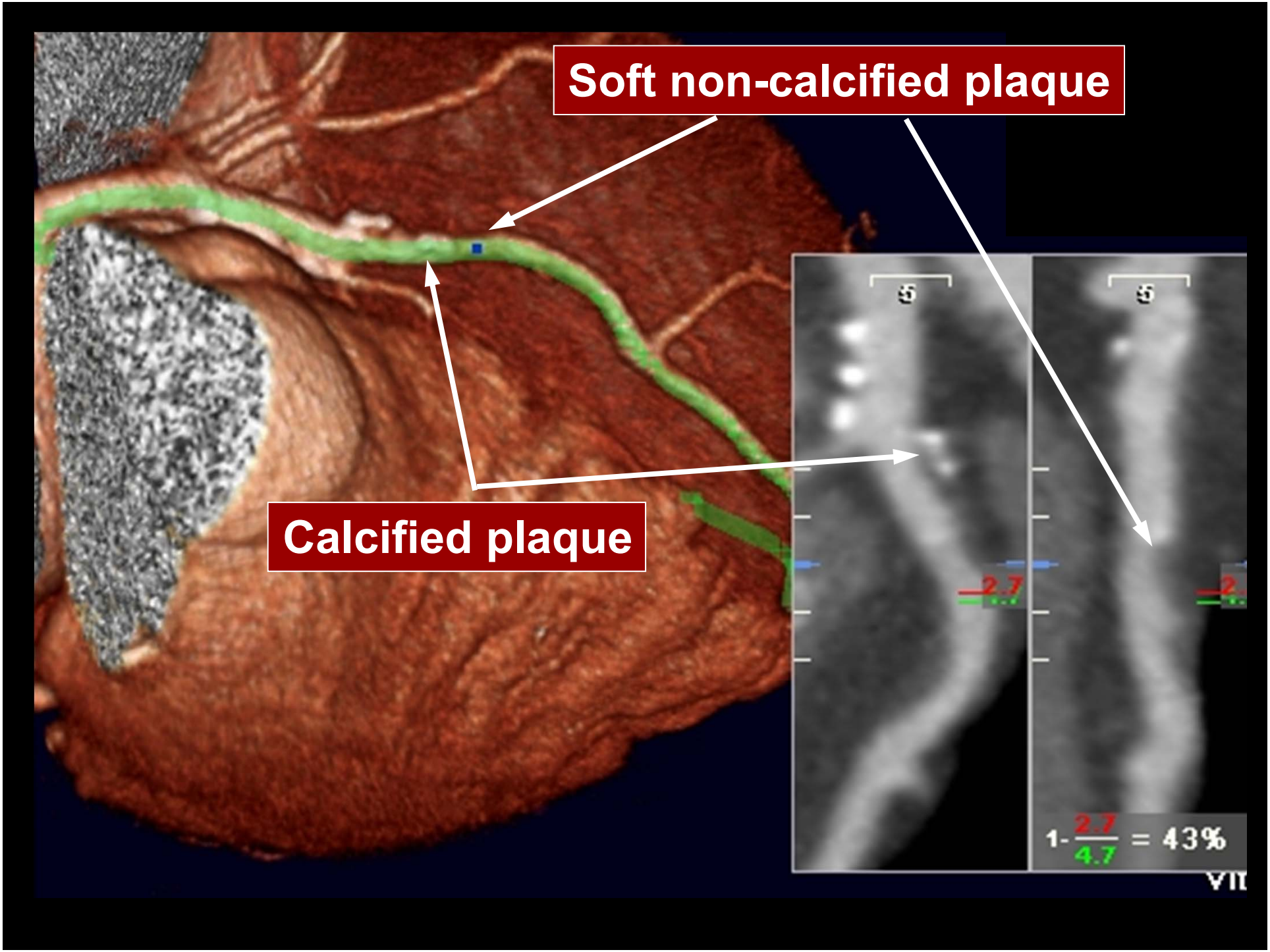
Western Diet + Salicylate



Tatjana Ignjatovic

TINSAL-CVD Trial Design (NHLBI funded)




Soft non-calcified plaque
Calcified plaque
1- 2.7/4.7 = 43%

Why Salicylic Acid (SA) is an Attractive Therapeutic Approach in Man

In plants

- SA is present in every plant higher than moss
- SA controls ‘systemic acquired resistance’ in plants, the defensive response to biotic (infectious) as well as abiotic (*e.g.* thermal, oxidative, osmotic, heavy metal) insults and challenges.
- During a stress response its operative concentration range is 0.1 – 0.5 mM
- Transcription factors (NPR1) and genomic responses are well characterized

Why Salicylic Acid (SA) is an Attractive Therapeutic Approach in Man

In humans

- SA has been in clinical use many decades to treat joint pain, and has a long and established safety profile
- Effective therapeutic concentration range is 0.5 - 2.0 mM.
- Transcription factors including NF-κB and genomic responses can be characterized

Hypothesis: Salicylate activates a systemic antistress response in animals as it does in plants

Why Salicylic Acid (SA) is an Attractive Therapeutic Approach in Man

Hypothesis: Salicylate upregulates the body's natural defense mechanisms

Potential conditions

- Insulin resistance and T2D
- Cardiovascular disease
- Neurodegenerative diseases (Alzheimer's, ALS)
- Sarcopenia, cardiac and skeletal muscle wasting
- Aging

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Jongsoon Lee
Diane Mathis
Markus Feuer
Alessandro Doria

BWH – HMS

Peter Libby
Galina Sukhova

TINSAL trials

TINSAL-T2D

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TINSAL-retina (NEI)
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