E 174 – DOE for Adipogenic Media Optimization

ng, 2023

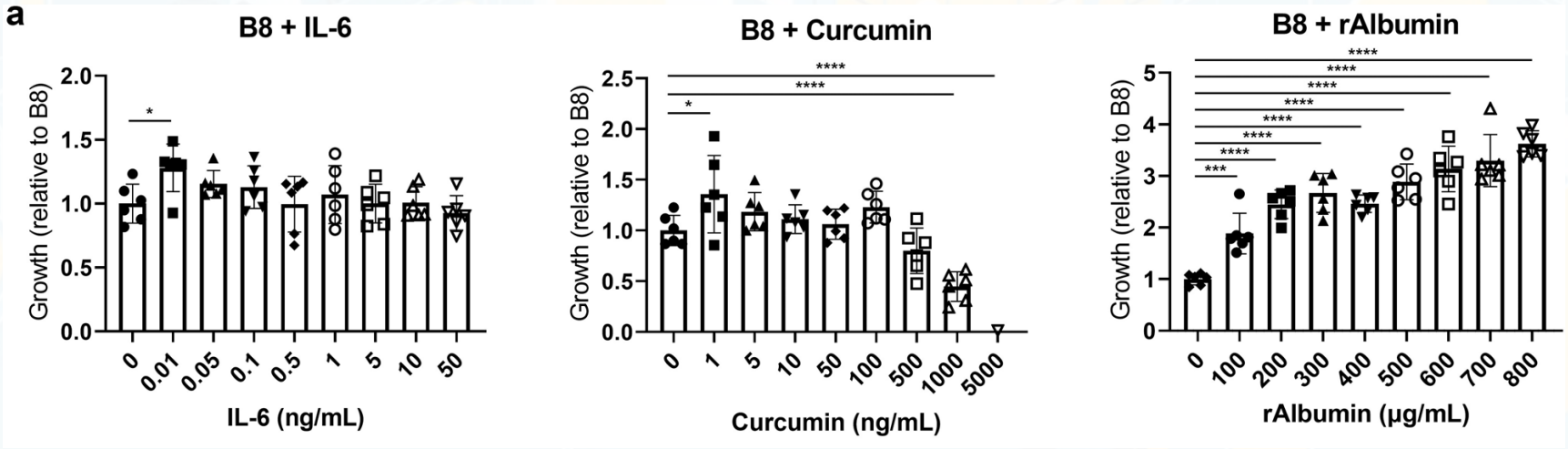
https://new-

Overview of Module 2

- Two objectives
  - To optimize the lipid accumulation media with design of experiments
  - To grow a larger amount of pig adipocytes then aggregate them into tangibly large cultured fat tissues/constructs
- Ideally, we'd first optimize media then use that for the fat tissue construct
- But because of time constraints, these two objectives will be performed separately

What are strategies to optimize media?

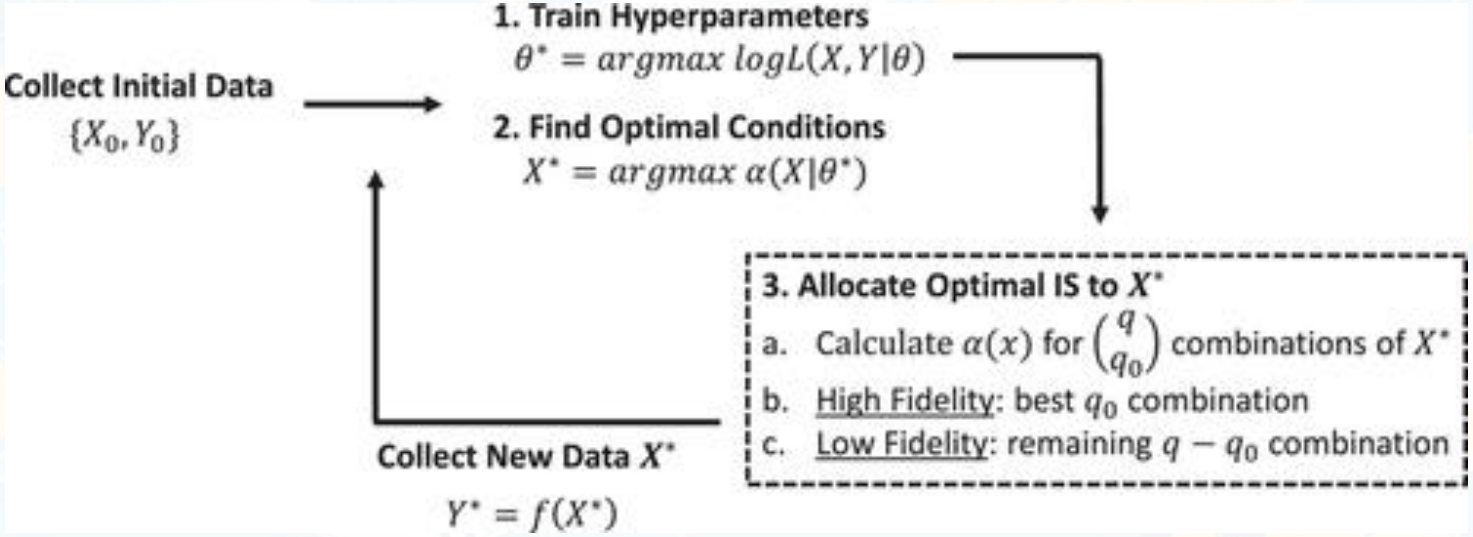
- One-factor-at-a-time (OFAAT)
  - Vary one factor (variable) while holding all others constant
  - Easy design and interpretation
  - Misses out on interaction between factors and is rather inefficient
    - → may miss the optimal set of conditions



Stout et al. 2022 – Beefy9

What are strategies to optimize media?

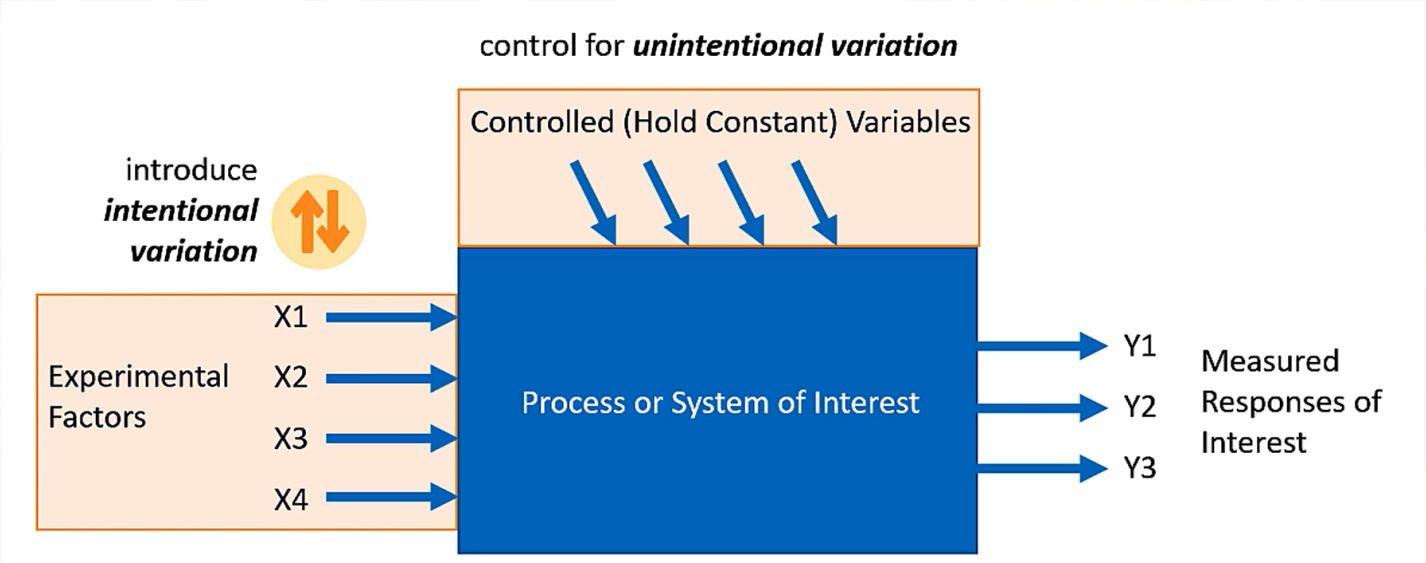
- Bayesian Optimization
  - Global optimization of “black-box” function
  - Complicated to understand



Cosenza et al. 2022. -- Multi-information source Bayesian optimization of culture media for cellular agriculture

What are strategies to optimize media?

- Design of Experiment (DOE)
  - Structured approach for data collection and making discoveries
  - Flexible in that you can choose the complexity of the design and thus interactions



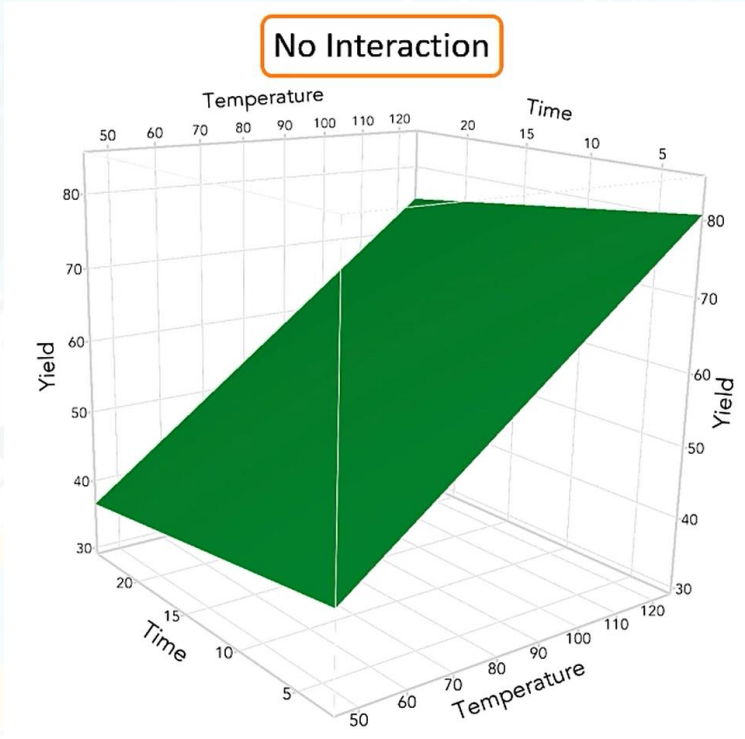
JMP statistical thinking for problem solving

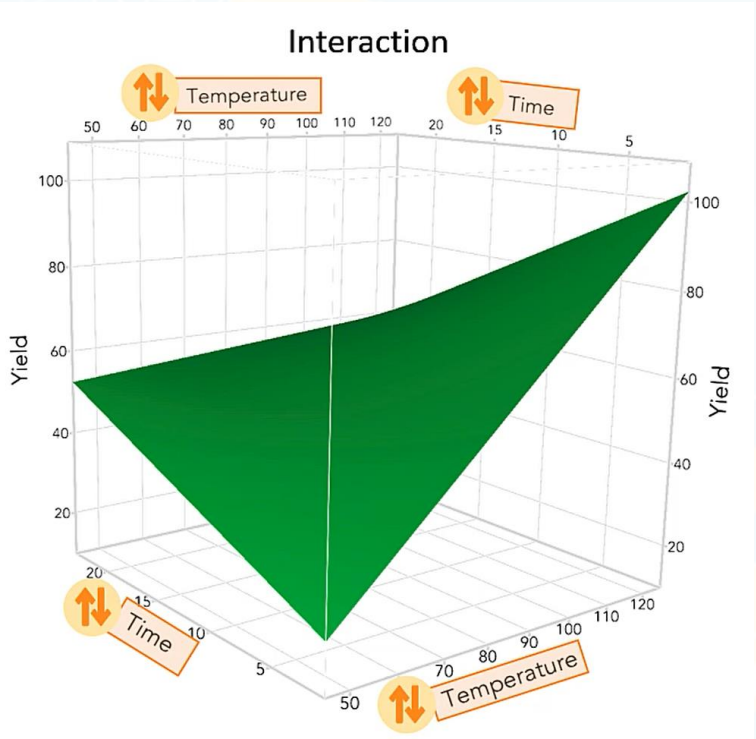
DOE: Interactions add curvature

Design space: Possible combinations of variables for experiments

Factors: Independent variables (experimental conditions)

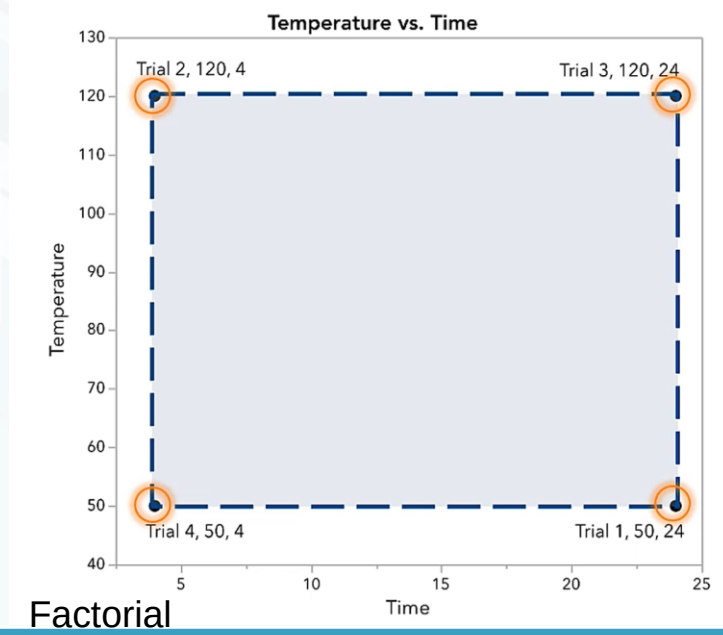
Response(s): dependent variables (what's measured)



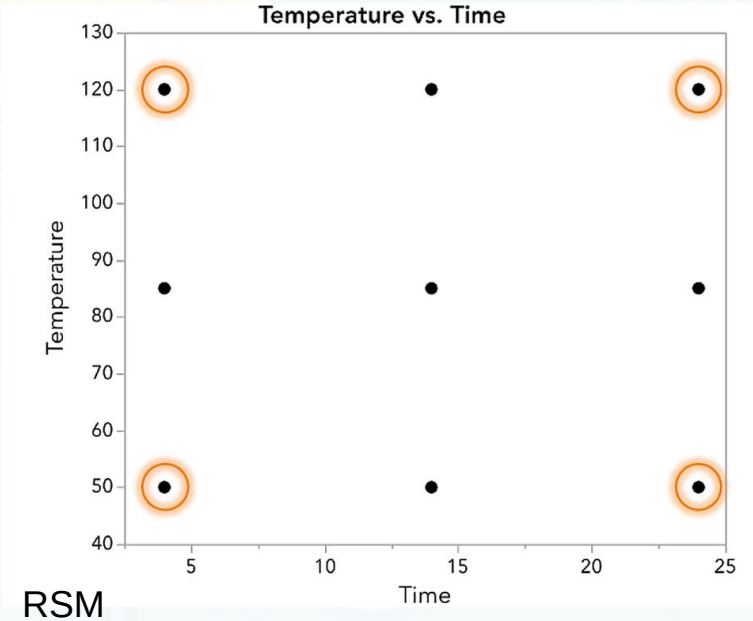


DOE Designs

- General process
  - Screening design – lots of factors, limited interactions
  - Full factorial design – every possible interaction, complicated with higher number of factors
  - Response surface methodology (RSM) – corners, “side” points, and center

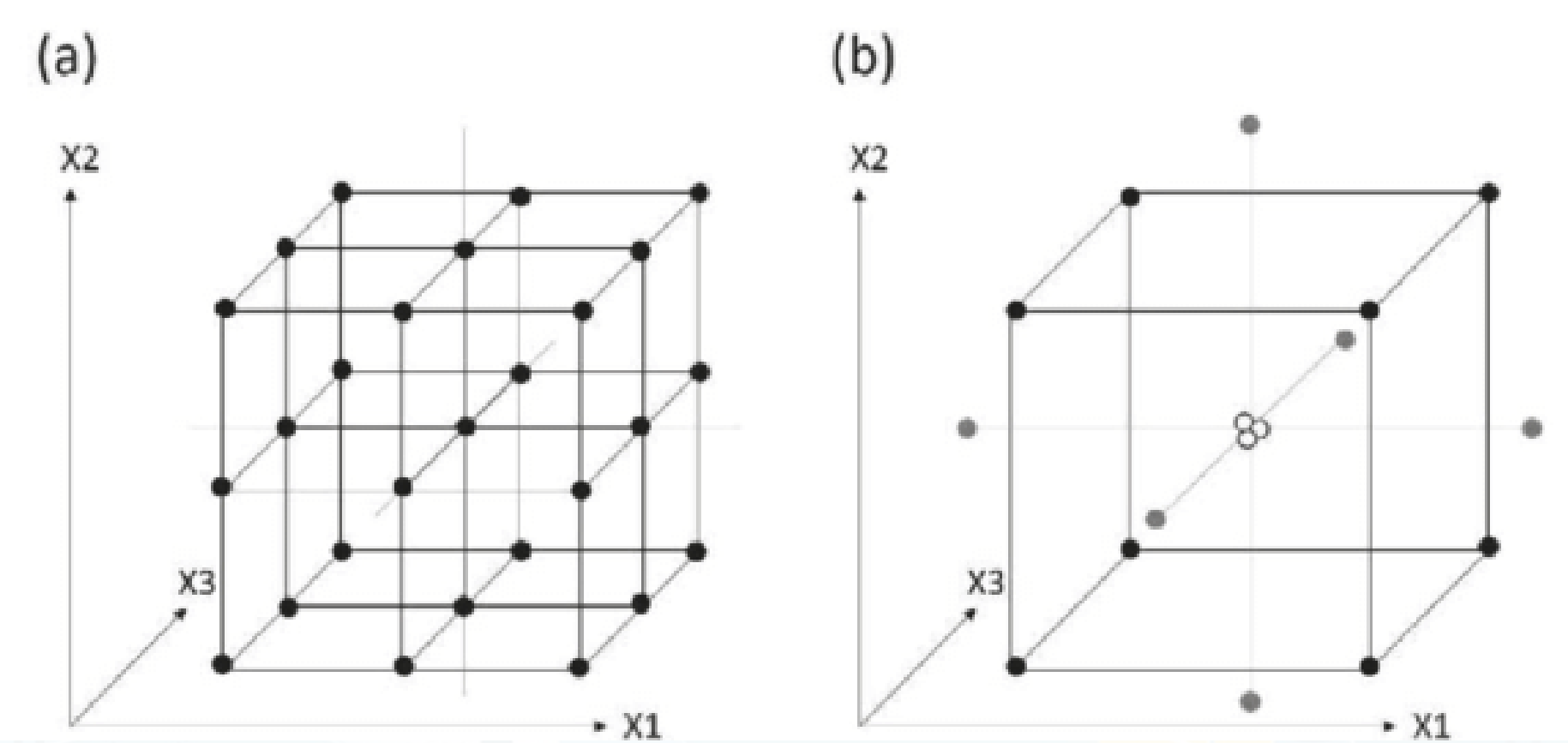


Factorial



RSM

Full factorial designs are more intense

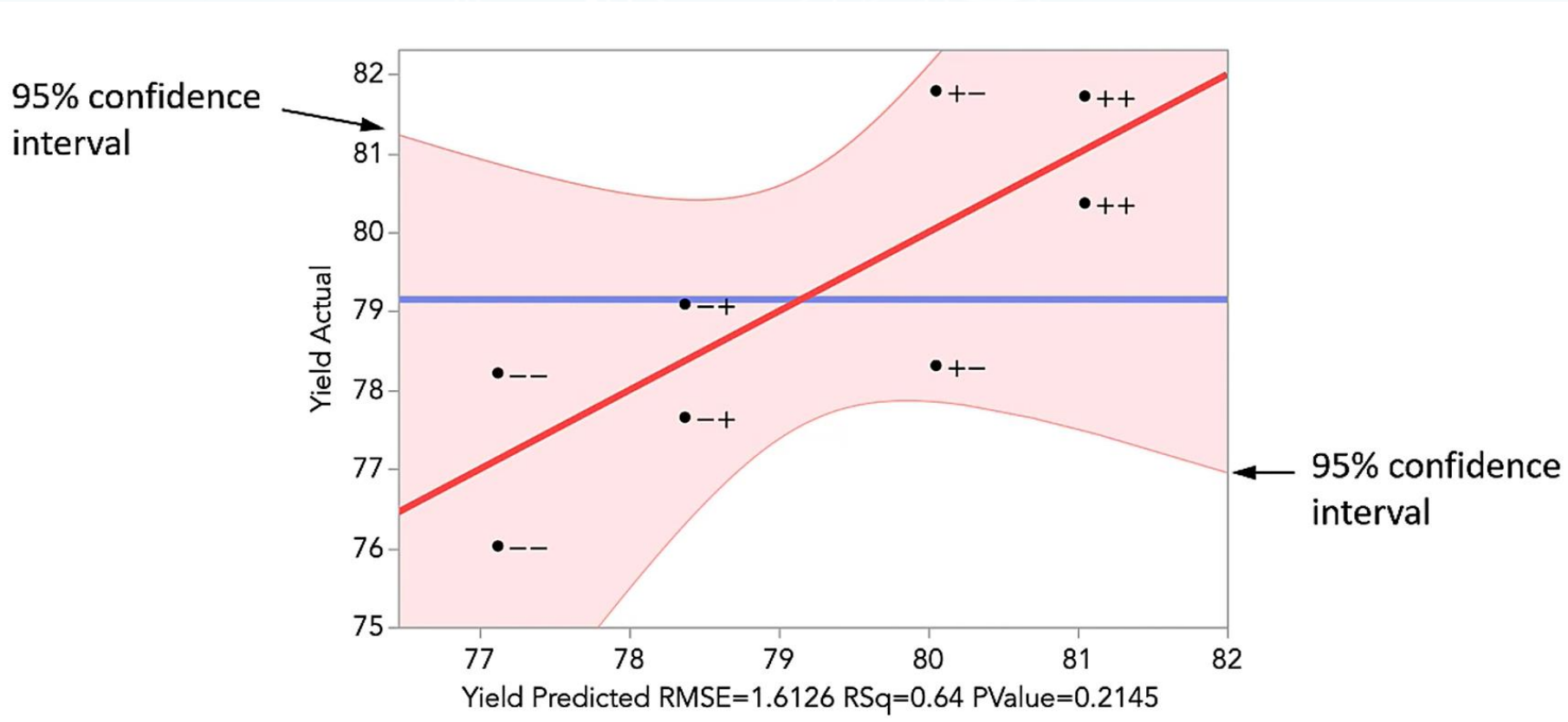


Full Factorial

RSM

DOE Analysis: Fit with model

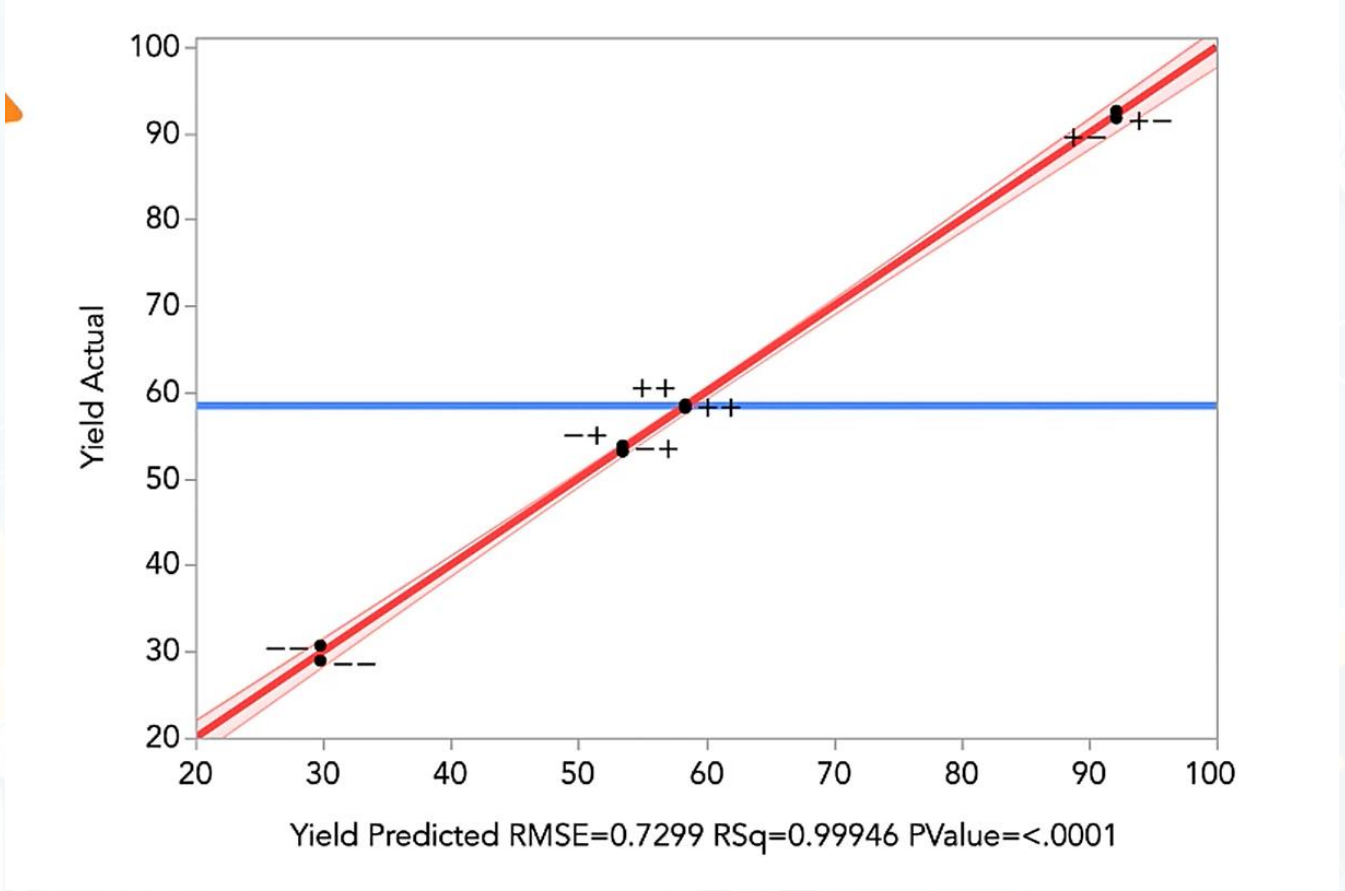
Poor fit



DOE Analysis: Fit with model

Good fit

We will be using standard least squares regression for our DOE analysis



Class DOE

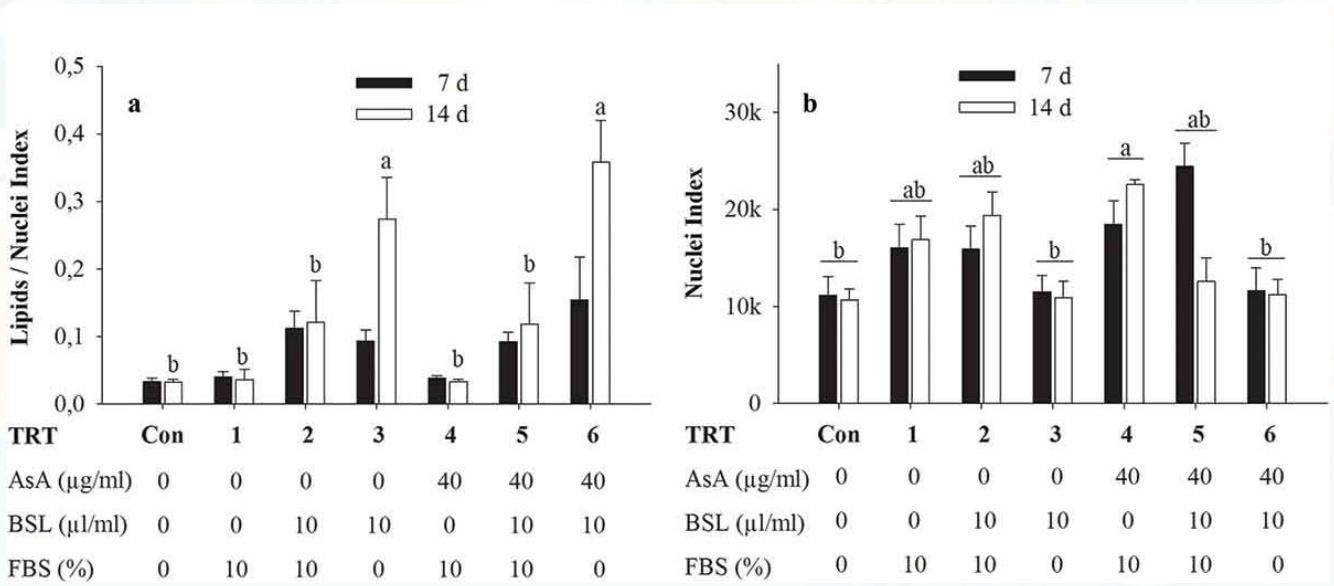
- As a team, we will contribute to a 4-factor DOE design
  - → 25 different media
- Every group will make 7 media (6 unique, one center point)
- We will be able to understand the interaction of these factors and model quadratic effects

Class DOE

- We will optimize the best performing lipid accumulation regimes from Module 1 – DM2 from Jurek *et al.*
- As a reminder of the formulations
  - **Induction Media** (2 Days): DMEM, 10% FBS, biotin (10 µM), pantothenate (5.67 uM), insulin (3 µg/mL), dexamethasone (0.3 µM), IBMX (0.1 mM), rosiglitazone (10 µM)
  - **Accumulation Media** (Rest of the time): DMEM, 0% FBS, insulin (3 µg/mL), biotin (10 µM), 113 uM ascorbic acid, 500 ug/mL Intralipid

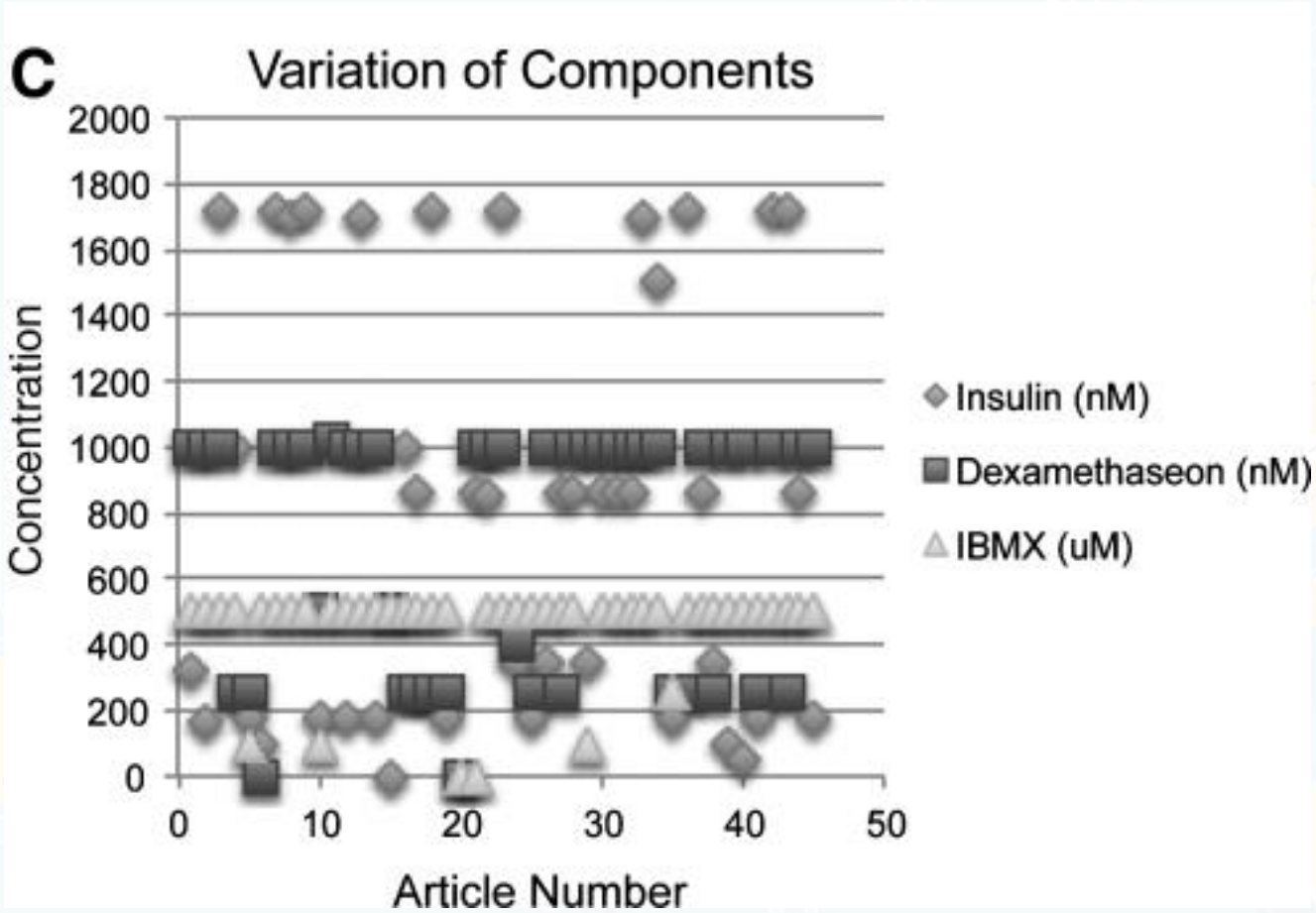
Class DOE

- We will optimize the DM2 accumulation medium
  - Jurek *et al.* tested ascorbic acid, bovine serum lipids, and FBS
  - We will test additives of pantothenate and IBMX
  - Also test varying concentrations of insulin and ascorbic acid



https://www.tandfonline.com/doi/full/10.1080/21623945.2020.1720480

Different Adipogenic Media Use Different Concentrations of Agonists

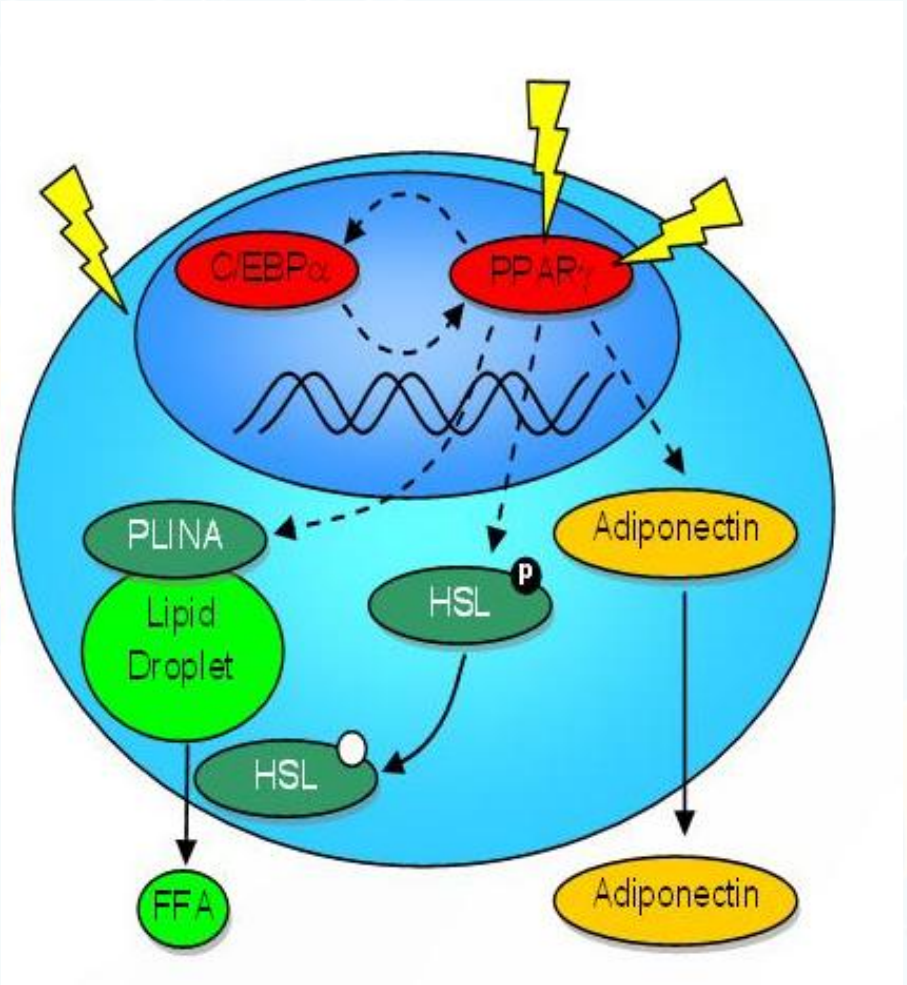


Which formulation is most optimal?

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3182038/

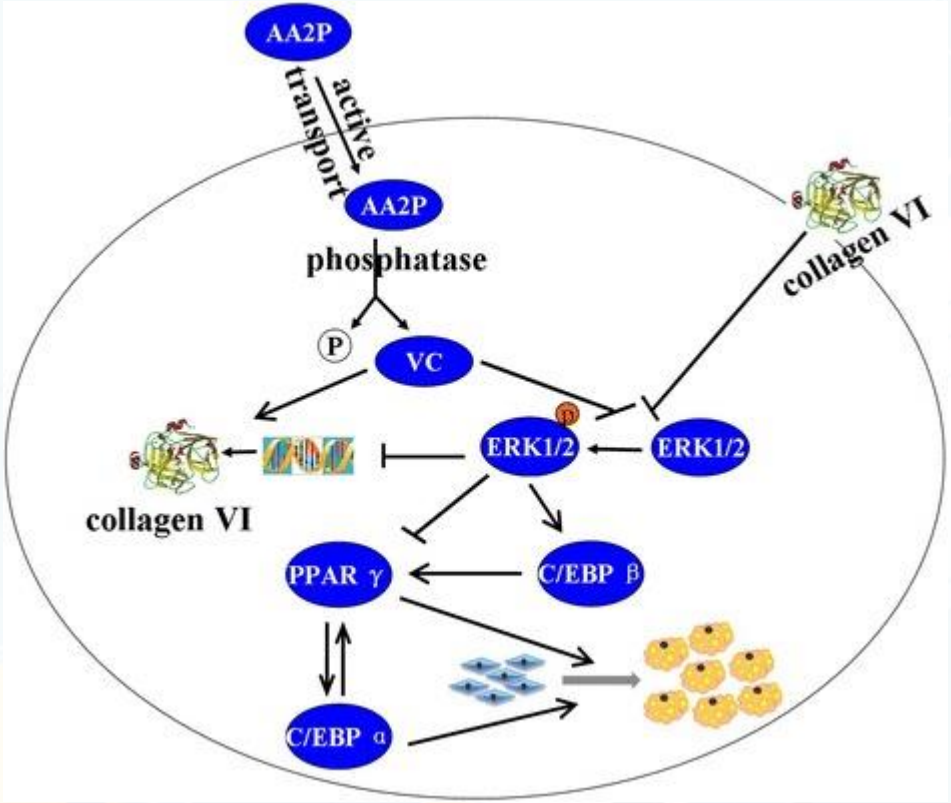
PPARγ is the master regulator of adipogenesis

- PPARγ directly or indirectly up-regulates hundreds of adipocyte-specific proteins, which include
  - Adipogenesis regulators (C/EBPβ)
  - Lipolysis regulators (HSL)
  - Secretory proteins (adiponectin)



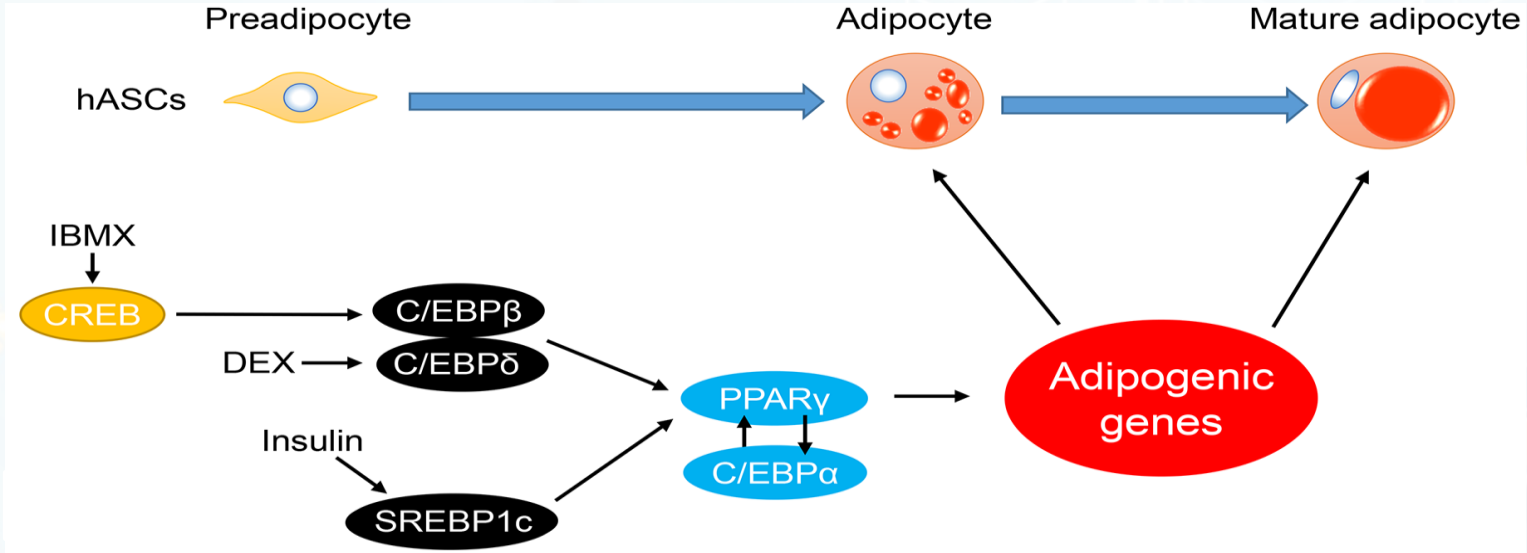
https://nanohub.org/resources/25395/watch?resid=25396

Ascorbic Acid



- Ascorbic acid treatment reduces ERK1/2 phosphorylation and increased collagen VI and adipogenic-specific factors
- ERK1/2 phosphorylation usually increase cell proliferation
  - ERK1/2 inhibits PPARγ
- Collagen VI enriched in mature adipocytes

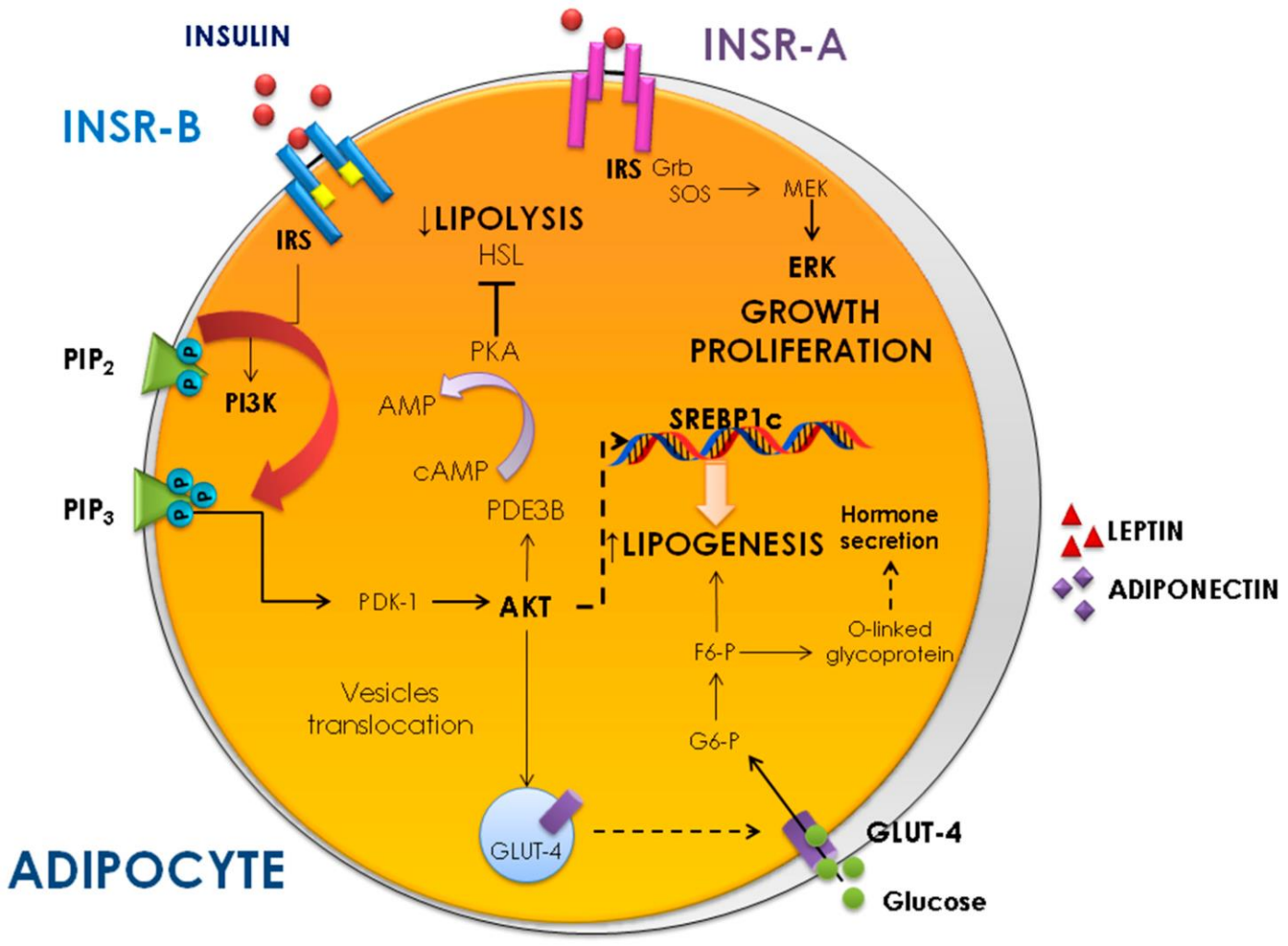
IBMX



- Competitive, nonselective phosphodiesterase inhibitor, raising intracellular cAMP and protein kinase A (PKA)
- Stimulates CREB which in turn activates C/EBPβ
- C/EBPβ then activates PPARγ, the master regulator of adipogenesis

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0192404

Insulin

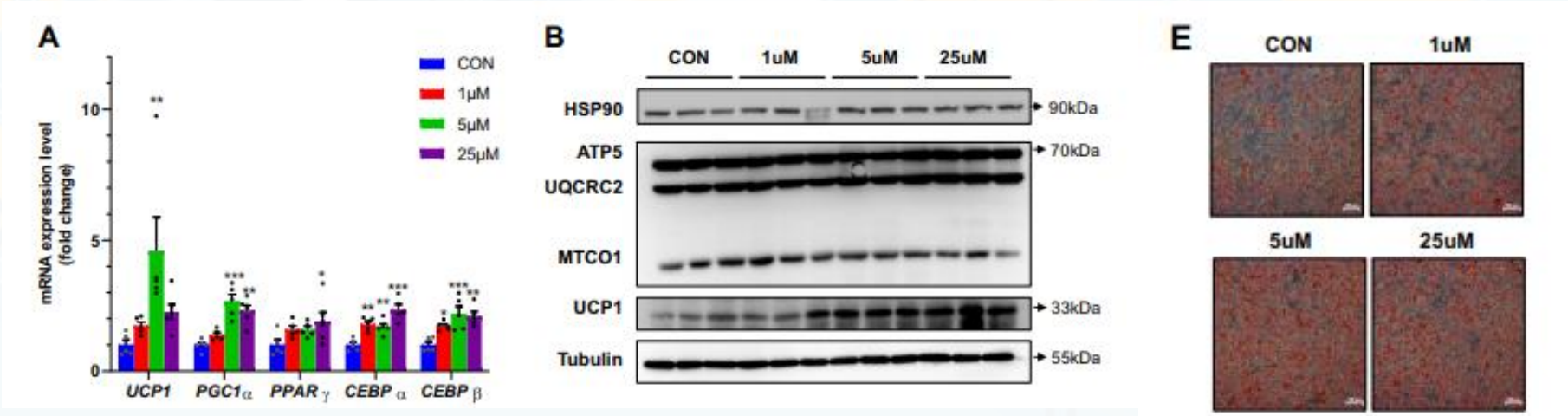


- Cross-talk with PPARγ
- Stimulates SREBP-1 and other transcription factors involved in adipogenic differentiation
- Limits lipolysis by inhibiting hormone-sensitive lipase (HSL)

https://www.mdpi.com/1422-0067/20/3/759

Pantothenate

- Associated with brown adipose tissue activation
  - Brown fat breaks down blood sugar (glucose) and fat molecules to create heat and help maintain body temperature
  - White fat stores energy
- Pantothenate leads to dose dependent increase of UCP1, mitochondrial carrier protein found in brown fat

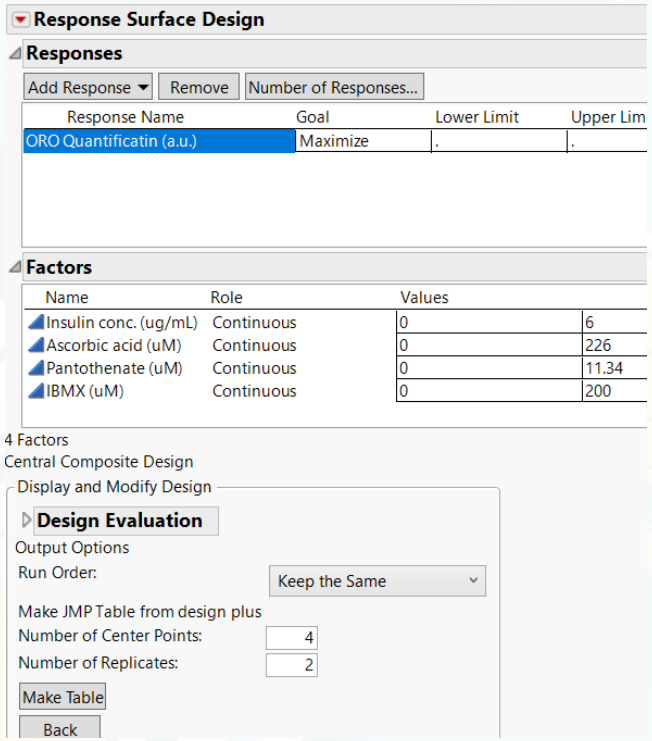


https://www.sochob.cl/web1/wp-content/uploads/2022/07/Pantothenate-protects-against-obesity-via-brown-adipose-tissue-activation.pdf

Response Surface Design

Made in JMP software

(Available for free through Tufts, but you don't need it for this class)



	Pattern	Insulin conc. (ug/mL)	Ascorbic acid (uM)	Pantothenate (uM)	IBMX (uM)	ORO Quantificatin (a.u.)
1	−−−−	0	0	0	0	•
2	−−−+	0	0	0	200	•
3	−−+−	0	0	11.34	0	•
4	−−++	0	0	11.34	200	•
5	−+−−	0	226	0	0	•
6	−+−+	0	226	0	200	•
7	−++−	0	226	11.34	0	•
8	−+++	0	226	11.34	200	•
9	+−−−	6	0	0	0	•
10	+−−+	6	0	0	200	•
11	+−+−	6	0	11.34	0	•
12	+−++	6	0	11.34	200	•
13	++−−	6	226	0	0	•
14	++−+	6	226	0	200	•
15	+++−	6	226	11.34	0	•
16	++++	6	226	11.34	200	•
17	a000	0	113	5.67	100	•
18	A000	6	113	5.67	100	•
19	0a00	3	0	5.67	100	•
20	0A00	3	226	5.67	100	•
21	00a0	3	113	0	100	•
22	00A0	3	113	11.34	100	•
23	000a	3	113	5.67	0	•
24	000A	3	113	5.67	200	•
25	0000	3	113	5.67	100	•
26	0000	3	113	5.67	100	•
27	0000	3	113	5.67	100	•
28	0000	3	113	5.67	100	•

24 media for corners and sides of tesseract (4D version of cube)

Each group's center point replicate

Your media optimization

	Pattern	Insulin conc. (ug/mL)	Ascorbic acid (uM)	Pantothenate (uM)	IBMX (uM)	ORO Quantificatin (a.u.)
1	----	0	0	0	0	•
2	---+	0	0	0	200	•
3	--+-	0	0	11.34	0	•
4	--++	0	0	11.34	200	•
5	-+--	0	226	0	0	•
6	-+-+	0	226	0	200	•
7	-++-	0	226	11.34	0	•
8	-+++	0	226	11.34	200	•
9	+---	6	0	0	0	•
10	+--+	6	0	0	200	•
11	+-+-	6	0	11.34	0	•
12	+-++	6	0	11.34	200	•
13	++--	6	226	0	0	•
14	++-+	6	226	0	200	•
15	+++-	6	226	11.34	0	•
16	++++	6	226	11.34	200	•
17	a000	0	113	5.67	100	•
18	A000	6	113	5.67	100	•
19	0a00	3	0	5.67	100	•
20	0A00	3	226	5.67	100	•
21	00a0	3	113	0	100	•
22	00A0	3	113	11.34	100	•
23	000a	3	113	5.67	0	•
24	000A	3	113	5.67	200	•
25	0000	3	113	5.67	100	•
26	0000	3	113	5.67	100	•
27	0000	3	113	5.67	100	•
28	0000	3	113	5.67	100	•

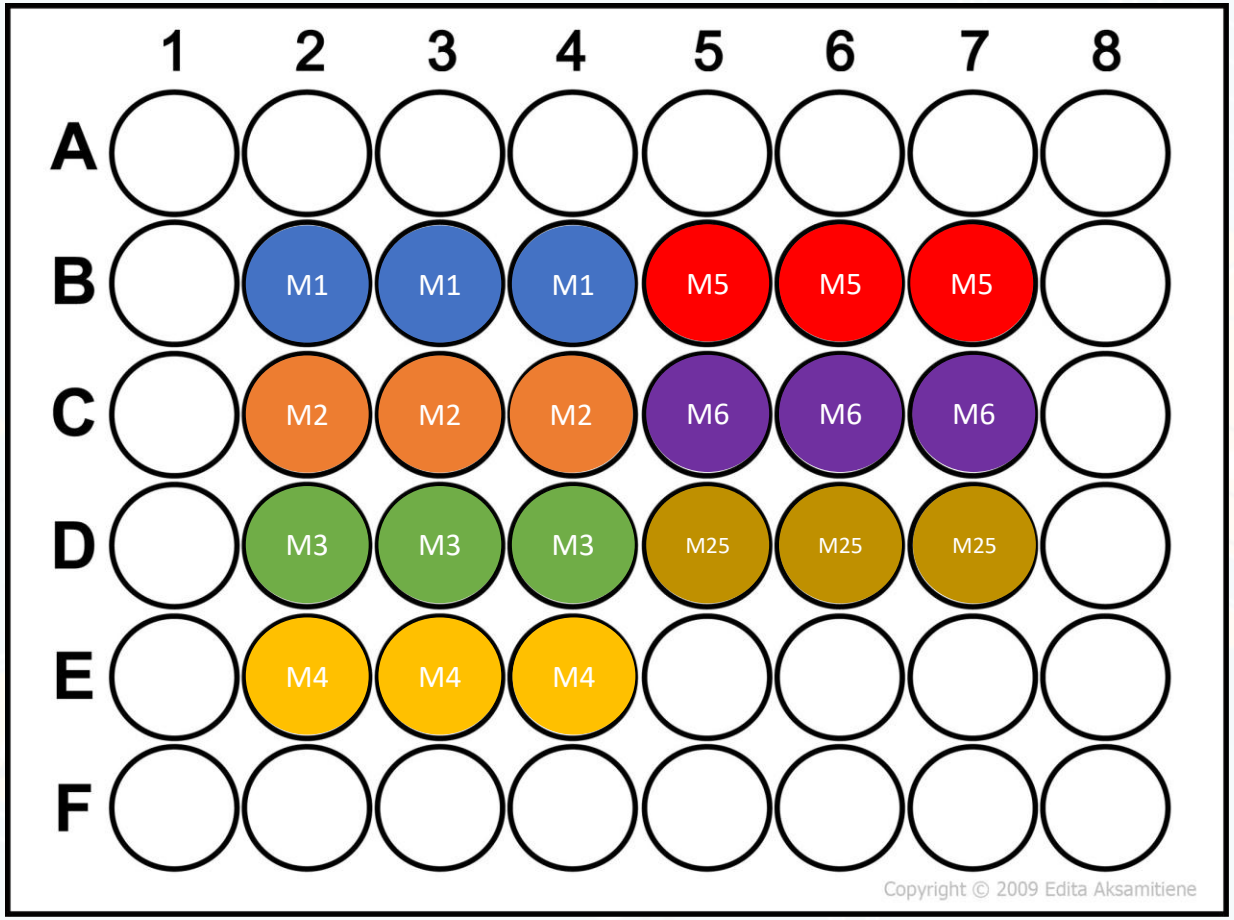
24 media for corners and sides of tesseract (4D version of cube)

Each group's center point replicate

+ 2 additional replicates

- You will make 7 media
  - 6 unique ones (we will assign)
  - 1 center point (repeated by all groups)
- -1/a: lower bound of the variable
- 0: middle between lower/upper
- 1/A: upper bound of the variable

Your well plate design



Different groups will be assigned different media

- Group 1: #1-6, 25
- Group 2: #7-12, 26
- Group 3: #13-18, 27
- Group 4: #19-24, 28

Module 2 Notes

- THAWING: POOLING ALL GROUPS' CELLS TOGETHER
- WE WILL USE PDFAT CELLS DURING THIS MODULE
- DO NOT USE THE MEDIUM THAT CONTAINS PUROMYCIN

What adjectives describe Module 2 / Lipid Accumulation?

- Fatty

(Optional Assignment) Module 2 Mixtape

Smooth like …

Decadent / Rich

Affectionate

Euphoric / Glutinous