# Investigating Menopausal Hormone Effects on Fat Metabolism

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## INTRODUCTION

**Background on menopause:**

- Menopause is the loss of ovarian function that leads to decline in sex hormones estrogen and progesterone. It is ensued by physiological changes and adverse symptoms that affect daily life[1].

**Relationship with cardiometabolic diseases:**

- Menopausal transition is associated with increase in harmful visceral fat in abdominal regions[2].
- Significant increase in fat accumulation and change in its distribution leads to obesity.
- Obesity increases risk of cardiovascular and metabolic diseases[3].

**Role of Hormone Replacement Therapy (HRT):**

- Combined oral HRT pills can increase concentrations of the declining hormones in blood.
- The symptoms of menopause may be subdued, including postmenopausal obesity[4].

**Objective of the research:**

- Find cellular-level data on the difference in lipid amounts in adipocytes under premenopausal and postmenopausal hormone concentrations.
- Test the effectiveness of HRT in offsetting the postmenopausal effects on lipid profile.

## METHODS

This research was a controlled, in-vitro lab experiment which consisted of:

- Culturing human pre-adipocytes in growth media.
- Differentiating the pre-adipocytes into *mature* adipocytes by adding differentiation media.
- Treating the cells with different hormone conditions, resulting in eight groups:

| Condition |
|---|
| 1) Control |
| 2) High estrogen |
| 3) Low estrogen |
| 4) High progesterone |
| 5) Low progesterone |
| 6) High estrogen+progesterone (premenopausal) |
| 7) Low estrogen+progesterone (postmenopausal) |
| 8) HRT mimic (est. + prog. combined) |

  - High levels -> average mid-luteal serum concentrations in menstruating women.
  - Low levels -> average post-menopausal serum concentrations.
  - The HRT mimic uses combined HRT pill Bijuva as reference.
  - All solutions diluted in nutrition media.

- Carrying out an Oil Red O staining protocol-
  - Lipid droplets stained red by Oil Red O.
  - Adipocytes imaged under a microscope to visualize the lipid.
  - Dye extracted from inside the cells; absorbance measured at 500 nm (the absorbance is proportional to lipid amount).

- Statistical analysis using ordinary one-way ANOVA test and post-hoc Tukey's multiple comparisons test (which compared different pairs).

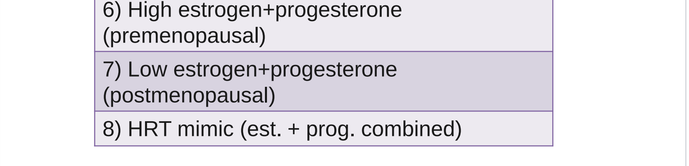


Figure 1: The summarized timeline of the execution of the method

## RESULTS

The result of growing and differentiating the preadipocytes is as follows:

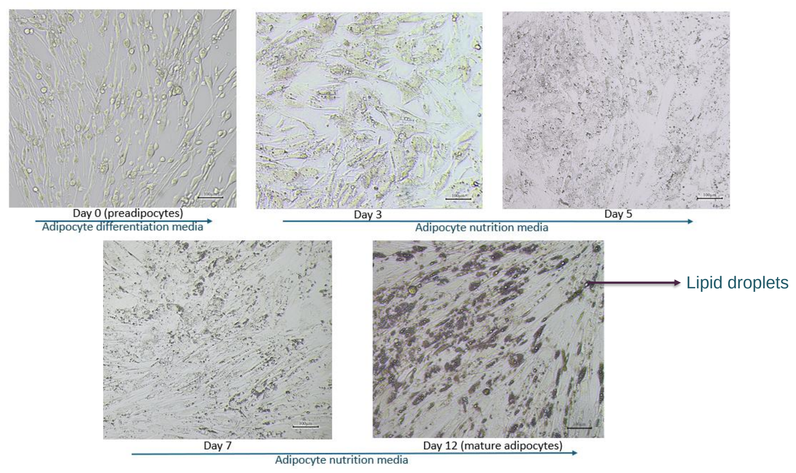


Figure 2: Timeline of adipocyte differentiation

- ANOVA confirmed that hormonal cell treatments had a significant effect on the lipid accumulation

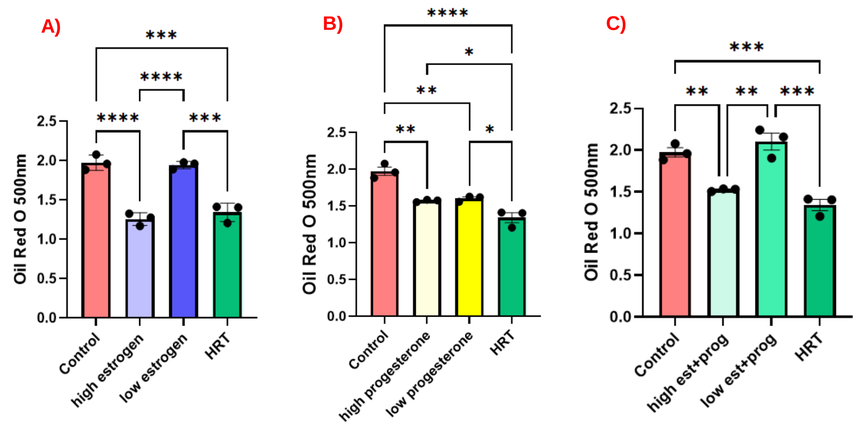


Figure 3. Bar charts comparing the mean absorbances of Oil Red O extracted from the different groups

- The post-menopausal group produced significantly higher mean absorbance than the pre-menopausal group.
  - This is evidence that lipid accumulation is higher in declined, postmenopausal hormone concentrations than pre-menopausal conditions.

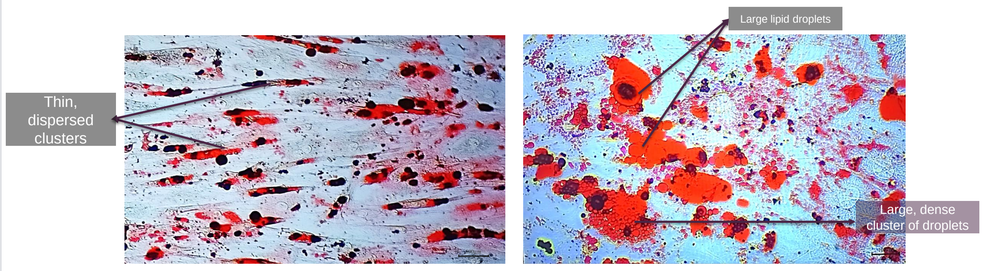


Figure 4. Oil Red O stained adipocytes of high est+prog condition (left) vs. low est+prog condition (right) at *20x magnification* under a microscope

- High concentration of estrogen had the most significant suppression on lipid accumulation in isolation.
  - The mean absorbance increases significantly in low concentration of estrogen (which reflects the baseline amount found in control).

- Progesterone treatment produced lower mean absorbances relative to control at both concentrations.
  - However, the presence of progesterone increased the mean absorbance in est+prog combination relative to individual estrogen only.

- The HRT mimic successfully decreased the lipid accumulation (low mean absorbance seen in Figure 3).
  - The lipid profile under HRT treatment had no significant difference from the premenopausal state.

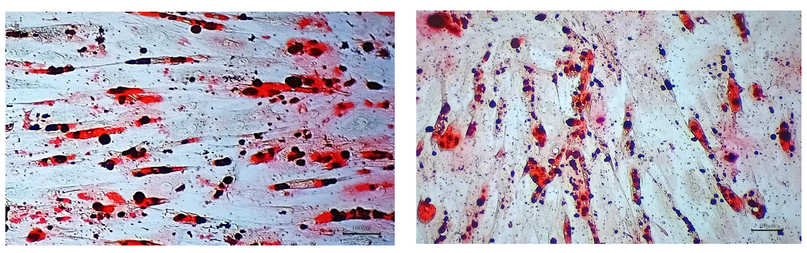

Figure 5. Oil Red O-stained adipocytes of high est+prog condition (left) vs. HRT mimic (right) at *20x magnification* under a microscope

## DISCUSSION

- Postmenopausal hormone conditions result in higher lipid accumulation than premenopausal conditions.
  - -> Evidence for the increase in postmenopausal obesity incidences.

- Estrogen may be the driver of lipid metabolism.
  - Supports previous literature that found estrogen to have abilities to break down lipid[5].
- Isolated progesterone suppressed lipid accumulation, but possibly hindered estrogen action in combination.

- The results support the use of HRT pills (after weighing the risk in a patient) due to its efficacy in lowering lipid amounts inside the cells.

**Limitations:**

- Small sample size
- Natural progesterone not used
- Limited project duration
- Complete physiological condition not reflected in-vitro.

## IMPLICATIONS

- Mechanistic pilot data collected that complements population-level data, and can be used to support future studies.

- Replication of the experimental design required.

- Potential developments include investigating cellular mechanisms affecting fat metabolism e.g. fatty acid uptake assay, qPCR testing mRNA expression

- Further research could improve HRT timing or hormone dosing to specifically reduce postmenopausal obesity risk.

## REFERENCES

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