The influence of different obesity phenotypes on the oxidative stress response in the MCF7 breast cancer cell line

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Abstract

Systemic metabolic health is a critical determinant of cellular signaling and disease progression, yet the specific mechanisms linking adiposity to breast cancer risk remain under-investigated. Preliminary data indicate that serum from obese subjects is associated with a downregulation of the antioxidant response gene, NRF2, and its downstream targets, suggesting a compromised oxidative stress response. The objective of this study is to evaluate how excess adiposity in women, categorized by varied obesity phenotypes, influences breast cancer cell growth and oxidative signaling. MCF7 breast cancer cells will be treated with serum from four distinct metabolic phenotypes: healthy control (CON), normal weight obese (NWO), metabolically healthy obese (MHO), and metabolically unhealthy obese (MUO). Following treatment, reactive oxygen species (ROS) production will be quantified using a fluorescence-based oxidation assay (CellROX). We hypothesize that cells cultured in serum from obese subjects will exhibit significantly higher ROS levels. This research aims to establish a functional link between systemic metabolic health and oncogenic cellular outcomes, providing insights that could refine medical assessments and therapeutic interventions for breast cancer in the context of obesity.

Approach

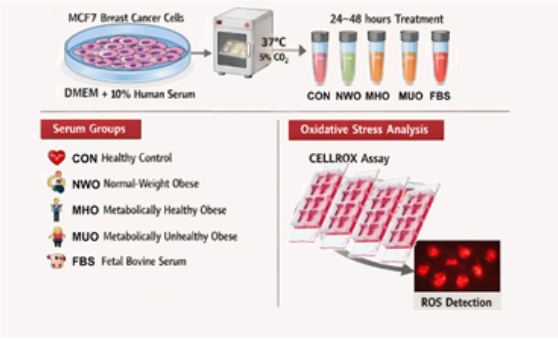


Figure 1. Experimental design for assessing oxidative stress in MCF7 cells treated with human serum. MCF7 human breast cancer cells are cultured in OMEM supplemented with 10% human serum under standard conditions (37 °C, 5% CO_2). Six treatment groups will be tested: serum from healthy control (CON), normal-weight obese (NOW), metabolically healthy obese (MHO), and metabolically unhealthy obese (MUO) donors (n = 3 per group, used individually), alongside fetal bovine serum. After 24–48 hours of treatment, intracellular reactive oxygen species (ROS) levels were measured using the CellROX fluorescence assay. Cells were co-stained with DAPI to visualize nuclei. Red fluorescence indicates ROS levels, normalized to DAPI signal.

Results

Serum from obese subjects reduces NRF2 activity in MCF7 cells

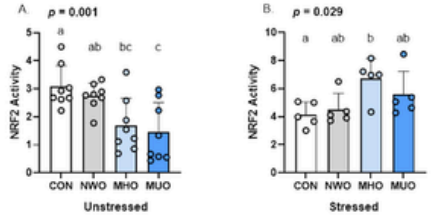


Figure 2. (A) Without exposure to a palmitic acid stressor NRF2 activity was highest in the group treated with CON human serum and comparable to the lower NRF2 activity of the NWO treatment group. Yet activity in the CON group was significantly higher than the MHO and MUO groups, with the MUO treatment group having the lowest NRF2 activity. When exposed to the high dose Palmitic acid,(B) NRF2 activity was lowest in the CON treatment group, although comparable to the NOW and MUO groups. Only the MHO treatment showed significantly higher NRF2 activity when compared to the CON group.

Obesity decreases NRF2 signaling in MCF7 cells

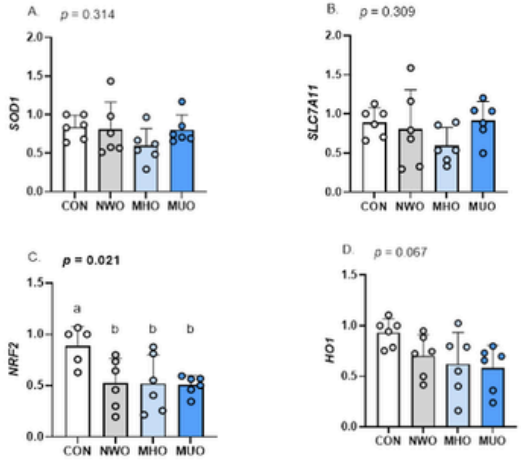
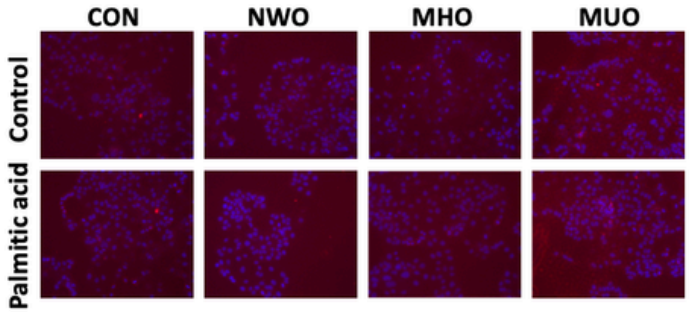


Figure 3. A major connection between obesity and cancer is that obesity-mediated metabolic perturbations are associated with increased oxidative stress and inflammation. As NRF2 is a key modulator of antioxidant signaling, we examined the mRNA expression of NRF2 and its downstream targets in MCF7 cells following their exposure to human serum from metabolically healthy, normal weight individuals (Con), as well as individuals with NWO, MHO, or MUO.

Results

Obesity does not influence basal ROS levels in MCF7 cells



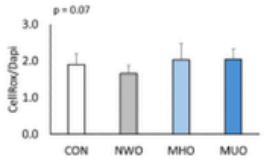


Figure 4. Increased ROS levels in MCF7 cells treated with obese serum and palmitic acid.
MCF7 cells were stained with DAPI (blue, nuclei) and CellROX (red, reactive oxygen species) following treatment with human serum from different metabolic phenotype.

Conclusions

- No significant effect on the level of reactive oxidation species was found

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