

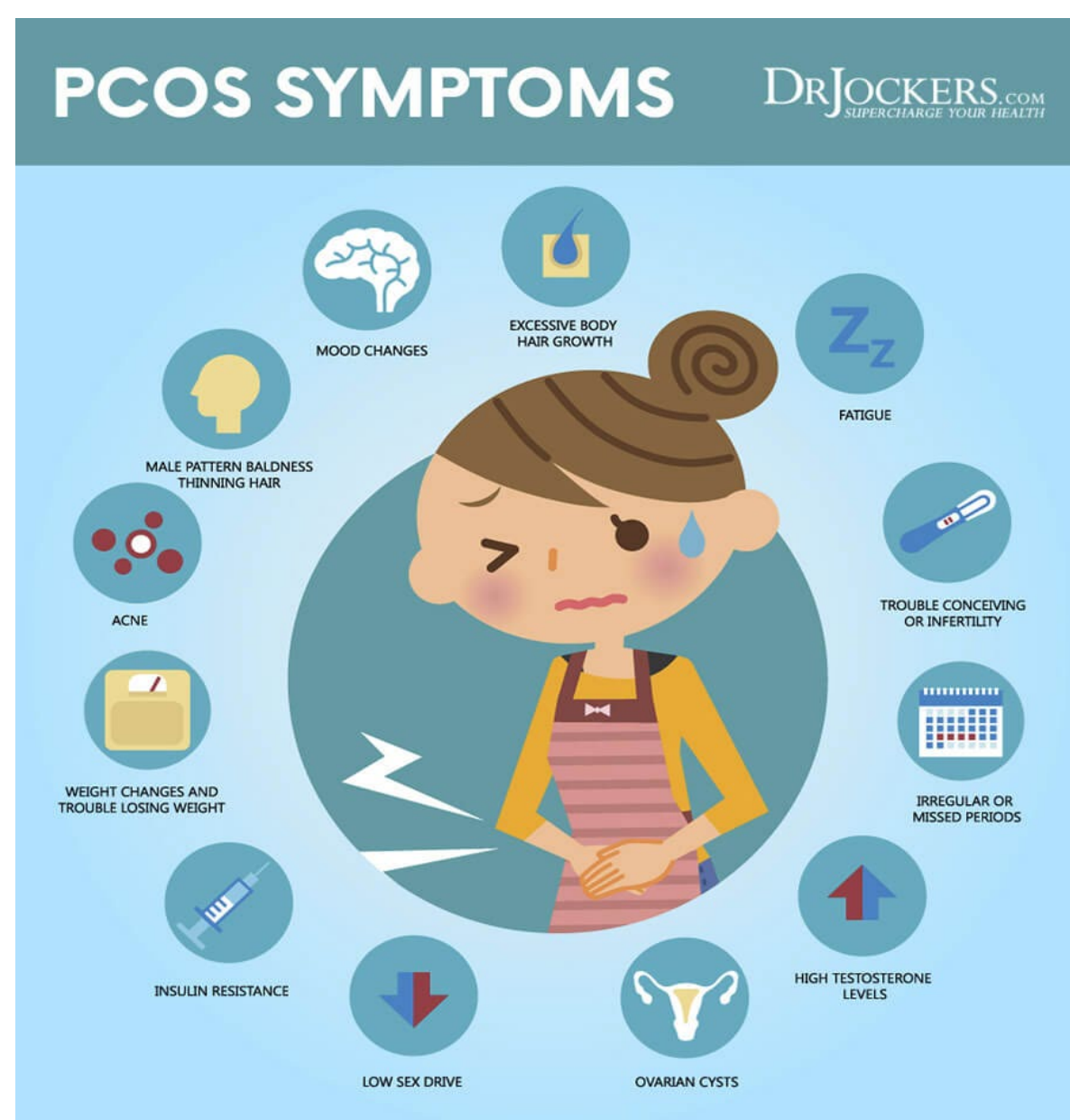
Effect of Whey Protein Metabolites on Insulin Signaling in women with Polycystic Ovary Syndrome (PCOS)

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Abstract

The purpose of this project was to examine the impact of whey protein (WP) ingestion on insulin sensitivity in women with and without polycystic ovarian syndrome (PCOS). Women with PCOS tend to have insulin resistance (IR), characterized by disrupted cell insulin binding resulting in reduced cellular glucose uptake. This faulty insulin response leads to high blood glucose levels. PCOS and non-PCOS women were administered oral glucose tests (OGTT) on Day 0 (baseline), Day 1 (start of WP treatment) and Day 7 (post-initiation of WP intake). Plasma insulin and glucose were measured. No significant difference in glucose responses for Day 0 and 1 was observed. Contrasting Day 0 to Day 1, insulin levels between the PCOS and non-PCOS women were significantly higher at 30 min after the start of WP treatment in the PCOS group and after 15 min and 90 min in the non-PCOS group. At Day 7, Non-PCOS insulin spiked at 15 min where PCOS women had a bimodal insulin response with peaks at 30 and 120 min. To further understand changes in insulin in PCOS vs. non-PCOS women before and after WP treatment, differentiated 3T3-L1 mouse adipocytes were treated with the human plasma collected during OGTTs for 48 hrs with media and cells were collected for protein and gene expression analysis. GLUT-4 expression was higher with 7th day plasma incubation in the non-PCOS women compared to Day 0 and to the PCOS woman at any time point. All GLUT-4 levels were elevated above the positive control cultures. While preliminary data identified minimal effects of whey protein supplementation on IR in PCOS women, acute WP treatment reduced peak glucose responses by enhancing insulin release in women with and without PCOS following glucose load.

Introduction



PCOS is an endocrine condition that affects up to 20% of females worldwide and is diagnosed by the presence of at least two of the following: increased androgen levels, irregular or absent menstrual cycles, and the presence of ovarian cysts (2). The diagnosis of PCOS is usually dependent upon the presence of at least two of the previously mentioned symptoms.

Fig 1: Symptoms of polycystic ovary syndrome (1).

Women with PCOS have abnormal metabolic functions, including inefficient use of insulin. Insulin resistance is characterized by a disruption in the insulin-binding process, potentially due to flawed intracellular insulin signaling, causing decreased cellular glucose uptake (2, 4). This faulty insulin response leads to increased blood glucose levels, diagnosed as type 2 diabetes. Moreover, increased blood insulin leads to more fat storage and lipid accumulation, as insulin promotes lipogenesis, leading to weight gain and difficulty in weight loss (5).

Methodology

Plasma Isolation and Collection: On Day 0 an oral glucose tests (OGTT) was administered to PCOS and non-PCOS women to identify possible basal differences in insulin levels between the two populations. On days 1 and 7 following initiation of whey protein ingestion, a second and third OGTT was administered. To plasma insulin response on Days 0 and Day 7 to the OGTTs was evaluated and the plasma was retained and stored at -80° Celsius.

Cell Culture: Adipocyte 3T3-L1 cells were plated in two 6-well plates, grown to confluency in Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and pen-strep (PSN) for five days. Cells were then differentiated in the aforementioned media with additional dexamethasone (DEX), 3-isobutyl-1-methylxanthine (MIX), and insulin. Differentiated for an additional 5 days.

Following differentiation, the cells were divided into three groups: cells treated with plasma from PCOS women, cells treated with plasma from non-PCOS women, and the original adipocytes treated with FBS as the control treatment. After a 24 hour exposure to the human plasma, the cells and the media were harvested.

Analysis: The cells were used for RNA extraction to perform a real-time PCR test, and the media was used to test for protein expression through an ELISA.



Fig 2: Whey protein (6).

Discussion & Conclusion

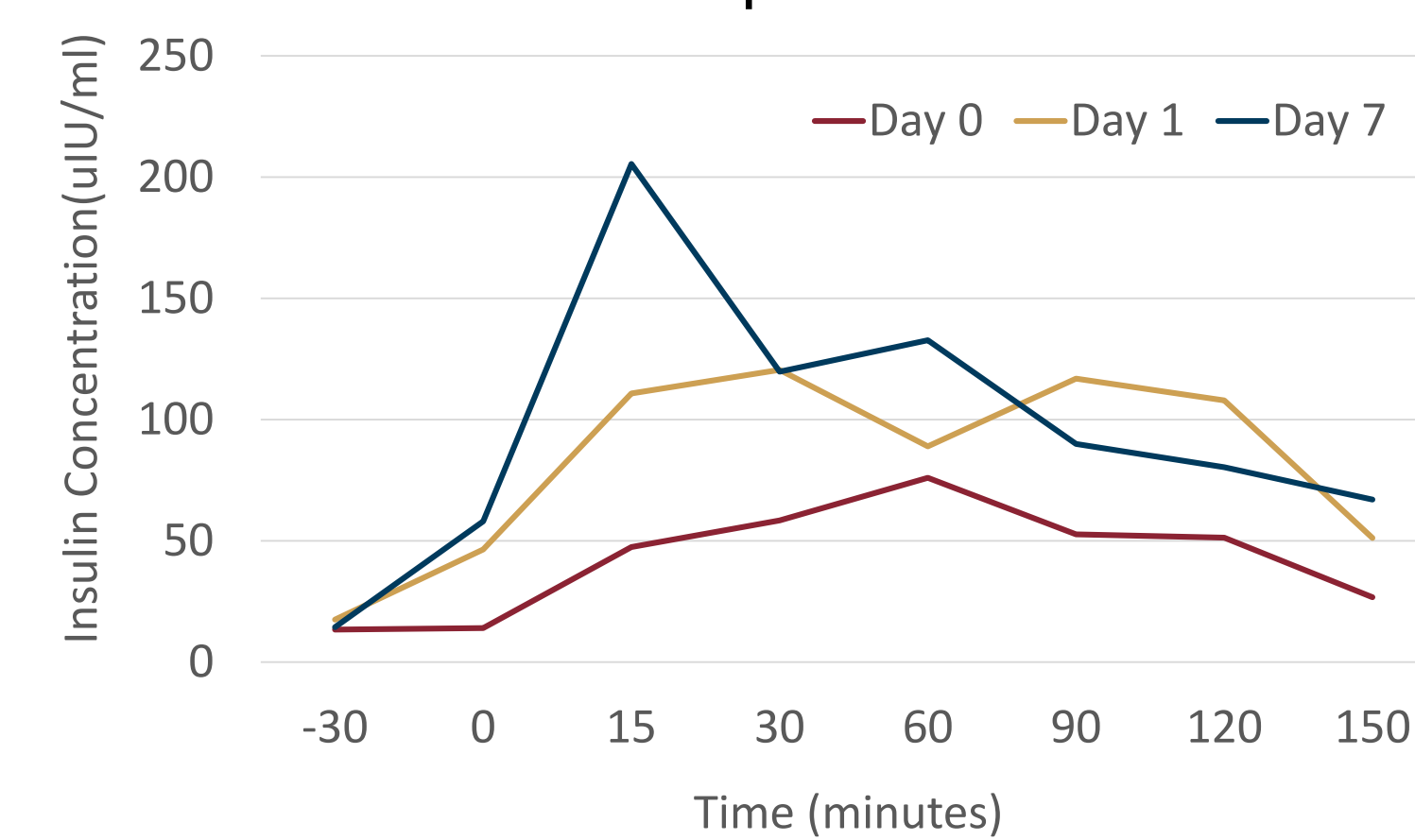
- There were significant differences in blood insulin concentrations between Day 0 and Day 7 in each group.
- PCOS Day 7 had a lower insulin spike, possibly indicating an impact of whey protein. Non-PCOS women had a higher Day 7 insulin spike that occurred sooner following glucose ingestion.
- The substantial increase in GLUT-4 expression from Day 0 to Day 7 in Non-PCOS women may be attributable to whey protein stimulation of the insulin signaling pathway.
- The nonsignificant difference between Day 0 and Day 7 in PCOS may be attributable to insulin resistance and that one week of whey supplementation may not provide an enough time to for any significant upregulation or downregulation of GLUT-4.
- This preliminary study may indicate that whey protein supplementation may be a useful method of influencing insulin resistance, as it appears to lend some control for insulin spikes following glucose ingestion.

Results

Insulin

Control Group:

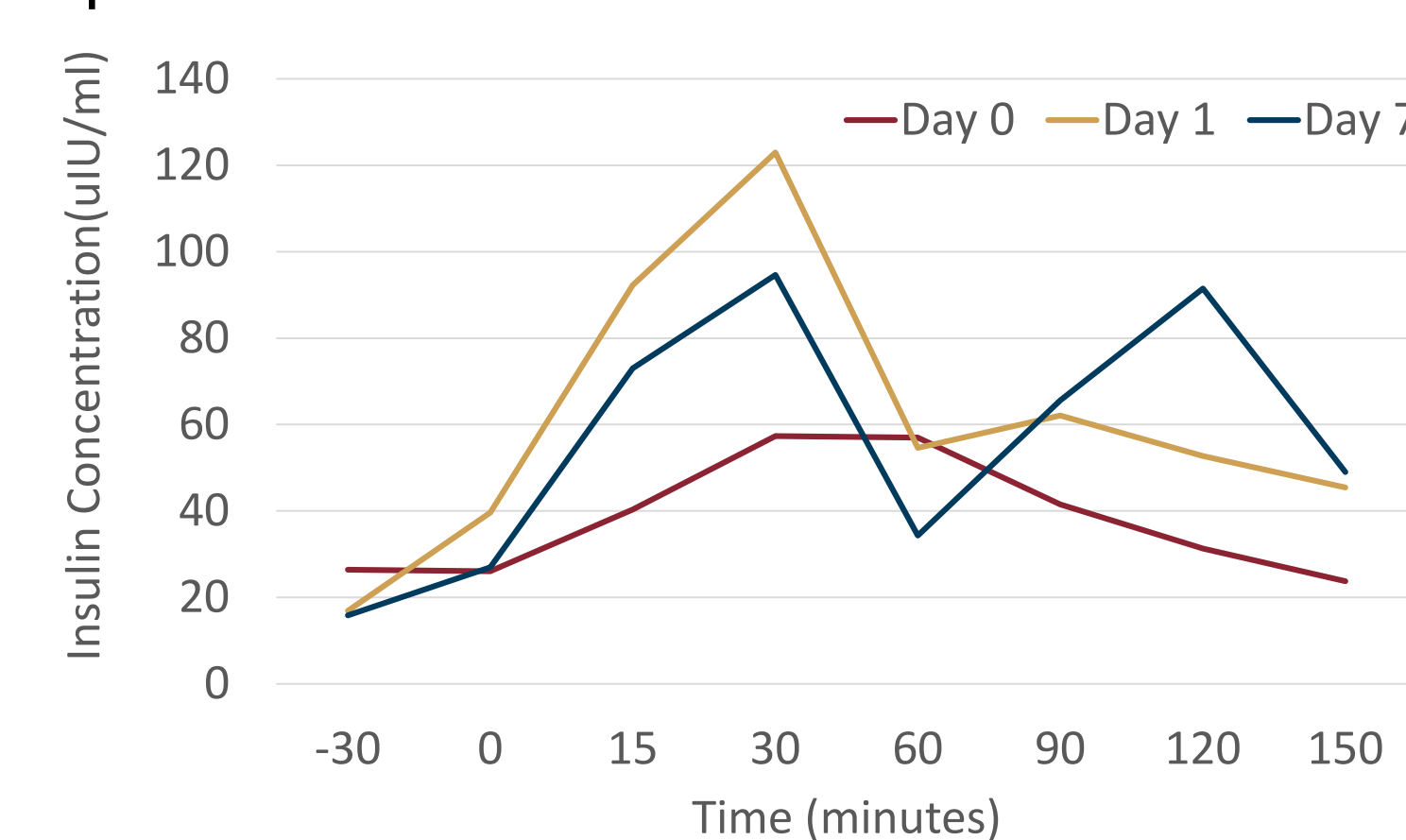
Differences in blood insulin concentration between Day 0 and Day 7 are significant, as shown below in Graph 1.



Graph 1: Insulin concentration vs. time in control group.

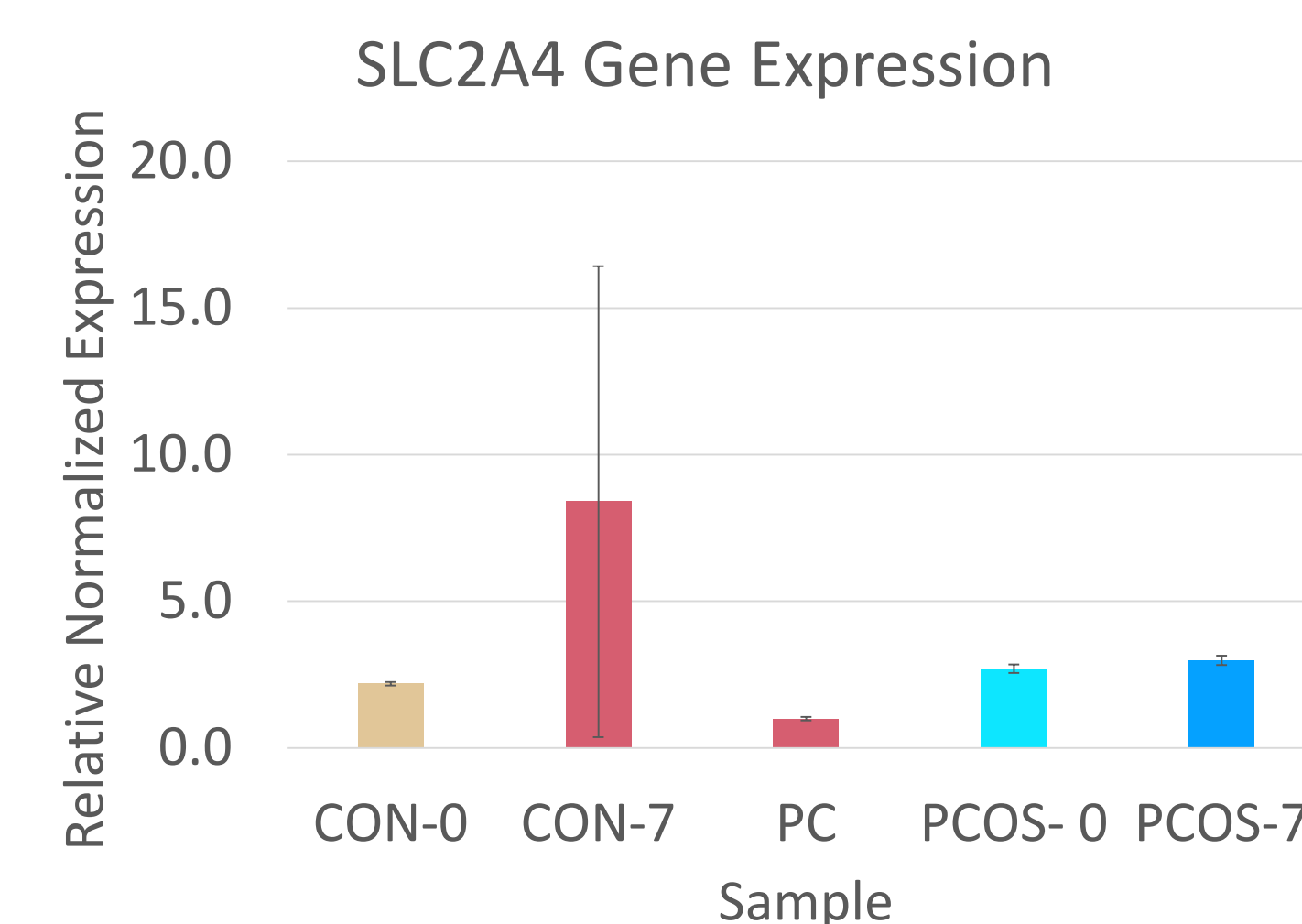
PCOS Group:

Differences in blood insulin concentration between Day 0 and Day 7 are significant, as shown below in Graph 2.

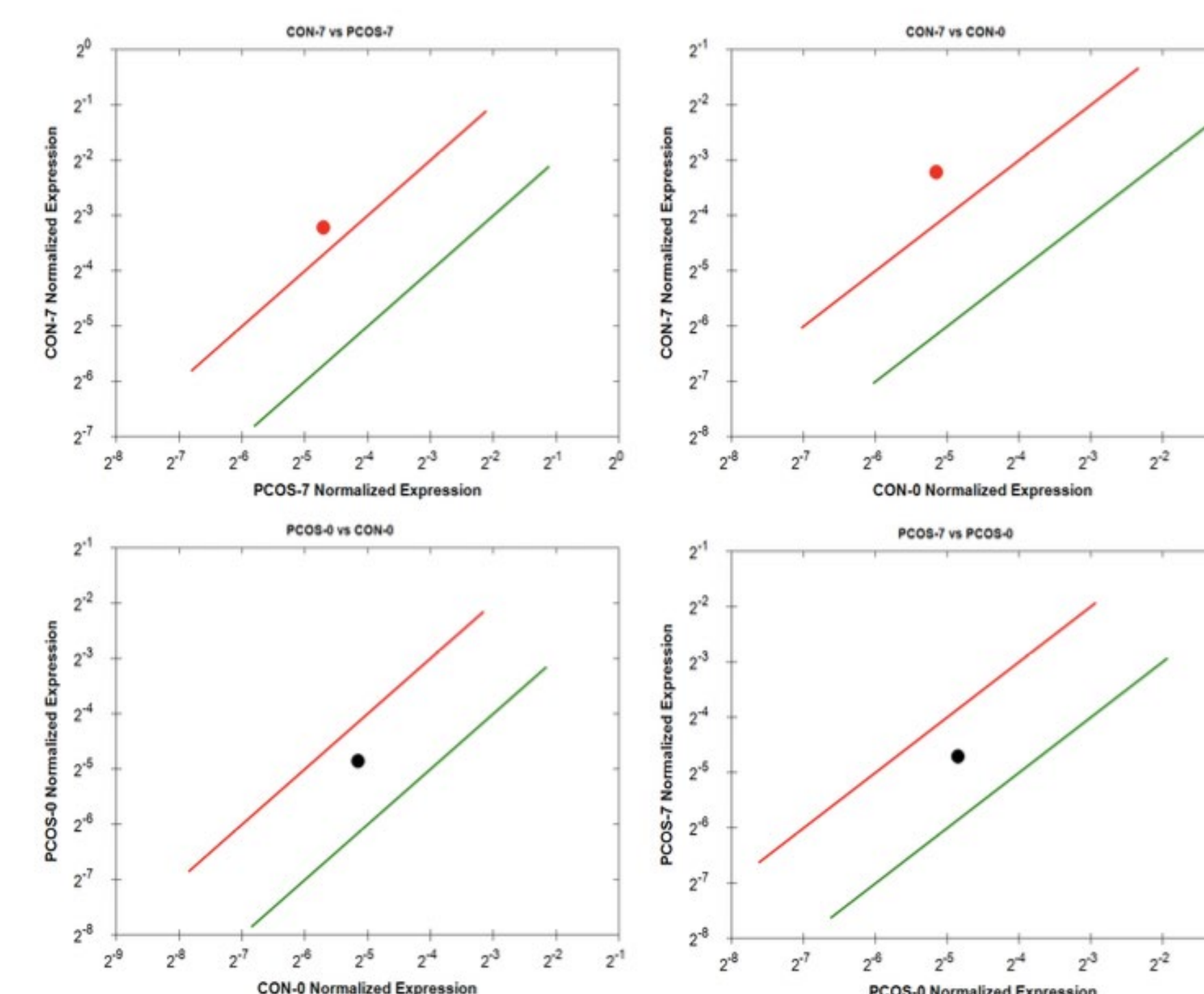


Graph 2: Insulin concentration vs. time in PCOS group.

GLUT-4 Gene Expression



Graph 3: Expression of GLUT-4 (SLC2A4) gene in control, PCOS, and positive control (PC) groups.



Graph 4: Comparison of expression of GLUT-4 (SLC2A4) gene in Control Day 7 vs. PCOS Day 7 (top left), Control Day 7 vs. Control Day 0 (top right), PCOS Day 0 vs. Control Day 0 (bottom left), and PCOS Day 7 vs. PCOS Day 0 (bottom right).

Further Studies

Possible further experimentation includes 40 day and 60 day studies that observe the insulin response and GLUT-4 expression after a longer period of time of whey protein supplementation.

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