Proceedings of the National Conference
On Undergraduate Research (NCUR) 2019
Kennesaw State University
Kennesaw, Georgia
April 11-13, 2019

Effect of Whey Protein Supplementation on Glucose Response and GLUT-4 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 the glucose response in women with and without polycystic ovarian syndrome (PCOS). Women with PCOS tend to have insulin resistance (IR), characterized by disrupted insulin binding resulting in reduced cellular glucose uptake and the consequential increase in blood glucose, or hyperglycemia. 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 glucose was measured. Changes in glucose from fasting levels were significantly lower on Day 1 compared to Day 0. To further understand changes in glucose metabolism in PCOS vs. non-PCOS women before and after WP treatment, differentiated 3T3-L1 mouse adipocytes were treated with human plasma collected during OGTTs following a 12 hour fast. Cells were cultured for 48 hours with plasma, after which, cells were collected for protein and gene expression analysis. GLUT-4 expression was higher with 7th day plasma incubation in the non-PCOS woman compared to Day 0 and to the PCOS woman on any test day. All GLUT-4 levels were elevated above the positive control cultures. While preliminary data identified minimal effects of whey protein supplementation on glucose and GLUT-4 responses in the participants, short- term WP treatment reduced peak glucose responses in women with and without PCOS following glucose load.

Keywords: Polycystic Ovary Syndrome, Glucose, Whey Protein

1. Introduction

Affecting up to 20% of women post-menarche worldwide, polycystic ovary syndrome (PCOS) is diagnosed by the presence of at least two of the three following criteria: increased androgen levels, irregular or absent menstrual cycles, and the presence of ovarian cysts[1]. Women with PCOS tend to have dysfunctional metabolism. This includes the inefficient use of insulin, a glucose-controlling hormone that is produced by pancreatic beta cells. Insulin resistance, commonly observed and implicated in the etiopathogenesis of PCOS[2], is characterized by a disruption in the insulin-binding process, causing erratic intracellular insulin signaling and decreased cellular glucose uptake in adipocytes, hepatocytes, and/or myocytes.[1,3] The insufficient postprandial insulin response leads to increased blood glucose levels diagnosed as type 2 diabetes. The pancreas increases insulin secretion to compensate for a lack of glucose uptake. As the pancreas continues to produce insulin to combat hyperglycemia, it becomes unable to meet the insulin demand and is eventually unable to produce sufficient amounts of insulin.[4] As insulin promotes lipogenesis, persistent compensatory hyperinsulinemia induces increased fat storage and lipid accumulation, leading to weight gain.[5]

Recent research suggests that insulin resistance could contribute to the onset of PCOS in some cases. GLUT-4, a principal insulin-stimulated glucose transporter that is coded for by the SLC2A4 gene, is responsible for the uptake of glucose into the cell and tends to be improperly regulated in women with PCOS. The activity of GLUT-4 in women with PCOS plays an impactful role in their body's overall response to glucose and can potentially contribute to insulin resistance. GLUT-4, which is primarily present in skeletal muscles and adipose tissue, is one of the most significant ways the body moves circulating glucose from the bloodstream into the cell.[6] GLUT-4, which is usually dependent upon the presence of insulin, functions by transporting glucose molecules from outside the cell into the cell, through the plasma membrane. A decrease in regulation of GLUT-4 or a decrease in GLUT-4 levels in peripheral tissue can contribute to insulin resistance, a characteristic of conditions such as type 2 diabetes and PCOS.[6] The expression of the SLC2A4 gene, whether it be downregulated or upregulated, impacts GLUT-4 activity in both polycystic and non-polycystic populations, the extent of which is being explored in this research. Additionally, abdominal obesity triggered by hyperandrogenism in women with PCOS, is also associated with low GLUT-4 expression.[6] It was hypothesized that whey protein or dietary protein would increase the expression of GLUT-4, allowing for greater cellular glucose uptake. In a PCOS populations, this would improve the response to insulin and decrease insulin resistance, ultimately allowing for lower blood glucose and reduced hyperinsulinemia. It is important to note, however, that androgen excess may be present in all women who have PCOS, whether they have insulin resistance or not.[7] And lastly, there are genetic and ethnic factors that play into insulin resistance. For example, Native Americans have an increased risk of becoming insulin resistant, resulting in type 2 diabetes onset.[2,8]

Several studies have established that whey proteins combat hyperglycemia in healthy individuals and patients with type 2 diabetes.[9-12] When a small dose of whey protein (preload) is ingested prior to a meal, it can significantly reduce blood glucose levels following the meal.[10] Additionally, whey's ability to curb one's appetite can also contribute to lower blood glucose levels.[10]

2. Objectives

The objectives of this research were to explore whether 7-day whey protein preload would decrease blood glucose levels and increase the regulation of GLUT-4 in women with and without PCOS.

3. Materials and Methods

3.1. Plasma Isolation And Collection

Four women with PCOS (PCO) and six age and ethnicity-matched control (CON) women with normal body mass index (BMI, 18.5 – 24.9 kg/m^2) were tested. On Day 0, a baseline 75 g oral glucose test (OGTT) was administered to both PCOS and CON women to identify possible differences in metabolic responses between the two groups. On the first and seventh days of 35 g whey protein (WP) preloading, a second and third OGTT were administered (referred to as Days 1 and 7 respectively). Plasma samples were isolated and stored at -80° Celsius.

3.2. Cell Culture

Fasting heparinized plasma from two different participants was used with one woman each from the control group and the PCOS group. 3T3-L1 adipocytes were seeded into 100mm plates and grown to confluence in 70% High Glucose Dulbecco's Modified Eagle's Medium (ThermoFisher Scientific, Waltham, MA), 10% fetal bovine serum (FBS, ThermoFisher Scientific), and 1% Penicillin-Streptomycin-Neomycin Antibiotic Mixture (ThermoFisher Scientific) for five days. The cells were then differentiated in the aforementioned media with additional 1 µM dexamethasone (Milipore-Sigma, St. Louis, MO), 0.5mM 3-isobutyl-1-methylxanthine (Milipore-Sigma), and 100nM insulin (Milipore-Sigma). They were then differentiated for an additional 5 days. Following differentiation, the cells were divided into five groups: cells treated with heparinized plasma from a PCOS woman (PCOS) on days 0 and 7, cells treated with heparinized plasma from a CON woman (CON) on days 0 and 7, and the original adipocytes treated with FBS as the control treatment (PC). After a 24-hour exposure to the human plasma, both the cells and the media were harvested.

3.3. Analysis

Following treatment, the 3T3-L1 cells were harvested and RNA was extracted using RNeasy Plus mini kit (Qiagen, Valencia, CA). Synthesis of cDNA was performed using iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad Laboratories, Inc., Hercules, CA). The expression of the GLUT-4 (SLC2A4) gene was measured by Quantitative Real-Time PCR using the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA). There were four replicates tested for each sample, with the exception of one sample, for which there were only two replicates. The GAPDH gene was used as a reference gene for normalization purposes. Analysis of mRNA expression data was performed using the CFX Maestro Software (Bio-Rad Laboratories). The media from the 3T3-L1 cells was also collected and used to test for insulin expression by ELISA (data not reported here).

A BioLis 24i chemistry analyzer (Carolina Liquid Chemistries, Greensboro, NC) was used to quantify glucose concentrations in participants' plasma. Each sample was run in duplicate and the average of the duplicates was used for graphical representation and statistical analyses.

4. Results

Participants in both PCOS and CON groups were premenopausal age-, BMI-, and ethnicity- matched women. Baseline characteristics (mean ± S.D) for both groups are detailed in Table 1.

Table 1. Characteristics of participants

	CON (n = 6)	*PCO (n = 4)*
Age (yr)	22.5 ± 4.2	25.8 ± 7.2
Weight (kg)	63.0 ± 5.5	59.9 ± 10.4
Height (in)	63.8 ± 2.7	64.0 ± 5.2
Waist circumference (cm)	81.1 ± 6.3	77.5 ± 11.7
Waist : Hip ratio	0.8 ± 0.1	0.8 ± 0.1
Body mass index (kg/m^2)	23.9 ± 0.7	22.6 ± 1.4
Ethnicity • Asian • Hispanic • Caucasian	 n = 1 n = 1 n = 4	 n = 1 n = 1 n = 2
Fasting baseline glucose level (mg/dl)	96.4 ± 13.3	95.5 ± 6.5

Repeated measures ANOVA revealed statistically insignificant effect of PCOS status, day of OGTT, and time of draws on glucose levels ($p = 0.23$, $p = 0.68$ and $p = 0.69$ respectively).

While both CON and PCOS women showed the same elevation in blood glucose with the Day 0 OGTT (baseline), with both peaking at 30 minutes, the PCOS women demonstrated a more prolonged elevation in blood glucose (Fig. 1 and 2). However, both groups returned to fasting levels by the end of the test, i.e. 150 min. Though not statistical, possibly due to the low sample size in each group, this longer period of glucose elevation resulted in a bigger AUC for glucose in the PCOS women (Fig. 3). PCOS status and time did not have a significant effect on glucose change from fasting levels during OGTT ($p = 0.45$ and $p = 0.51$ respectively). These changes were significantly affected by Day of testing ($p = 0.045$). Changes in glucose levels were higher on Day 0 compared to those on Day 1 ($p = 0.033$) but not in comparison with Day 7 ($p = 0.613$). With whey protein supplementation, the plasma glucose response to the OGTT was attenuated in both PCOS and CON, with both groups demonstrating approximately the same pattern and blood glucose maximum on Days 1 and 7 ($p = 0.835$). Significantly, where the Day 0 OGTT differed between PCOS and CON women, with whey protein ingestion, the OGTT test on Days 1 and 7 were similar, even achieving the same maximum at 2 hours for both test groups, a maximum that was both lower and 1.5 hours delayed from the Day 0 OGTT max (Fig 1 and 2). Though not statistical, the change in the PCOS resulted in an AUC the same as the CON women, all of which were lower than the PCOS women on Day 0.

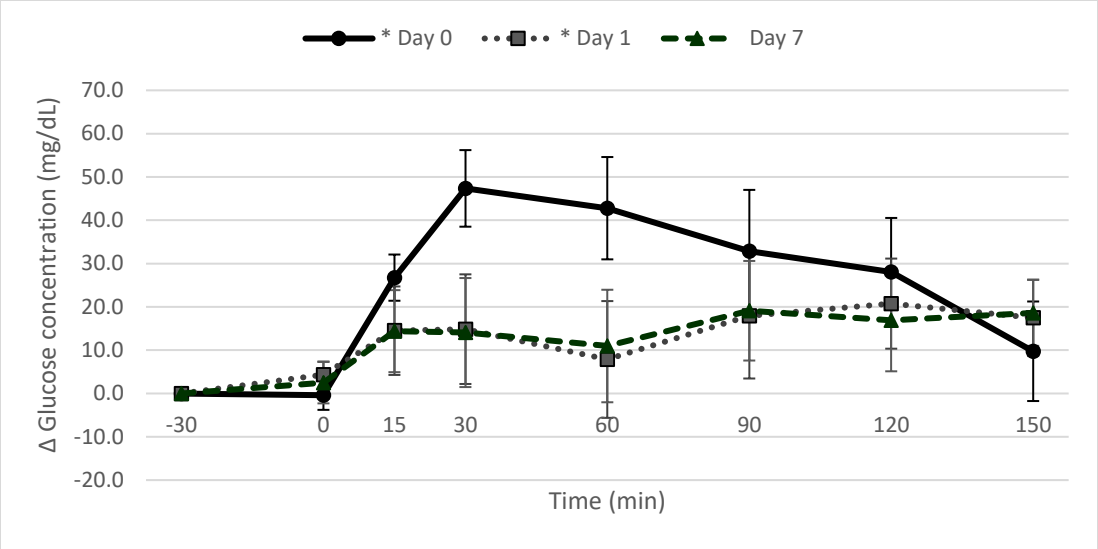

Figure 1. Mean (± SEM) changes in plasma glucose in PCOS group before and after 7-day supplementation (* denotes P<0.05)

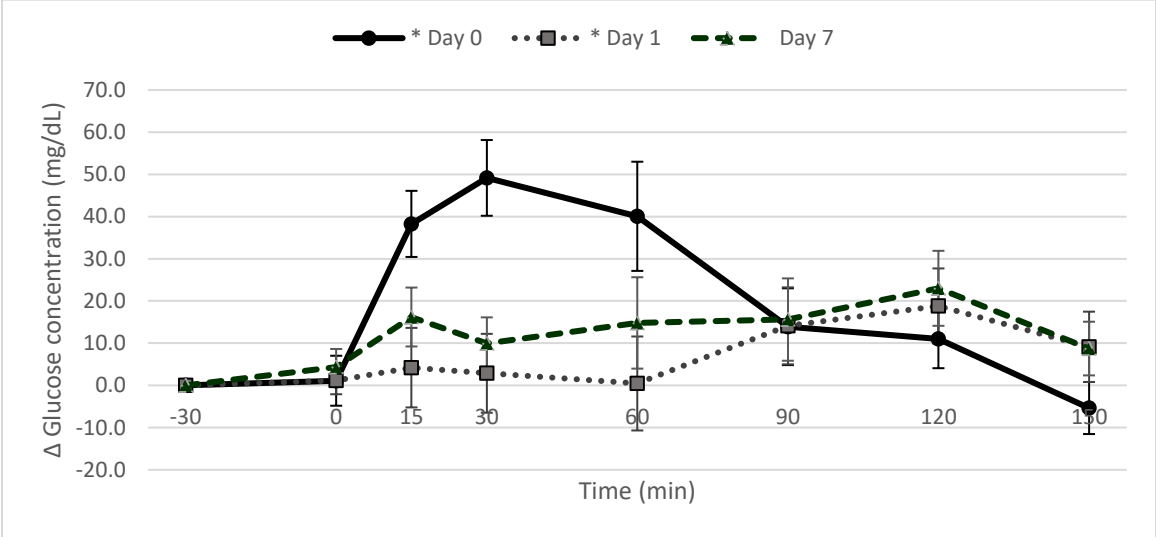

Figure 2. Mean (± SEM) changes in plasma glucose in CON group before and after 7-day supplementation (* denotes P<0.05)

Glucose AUC for Days 0, 1, and 7 OGTT were similar in both, PCOS and CON groups (p = .48).

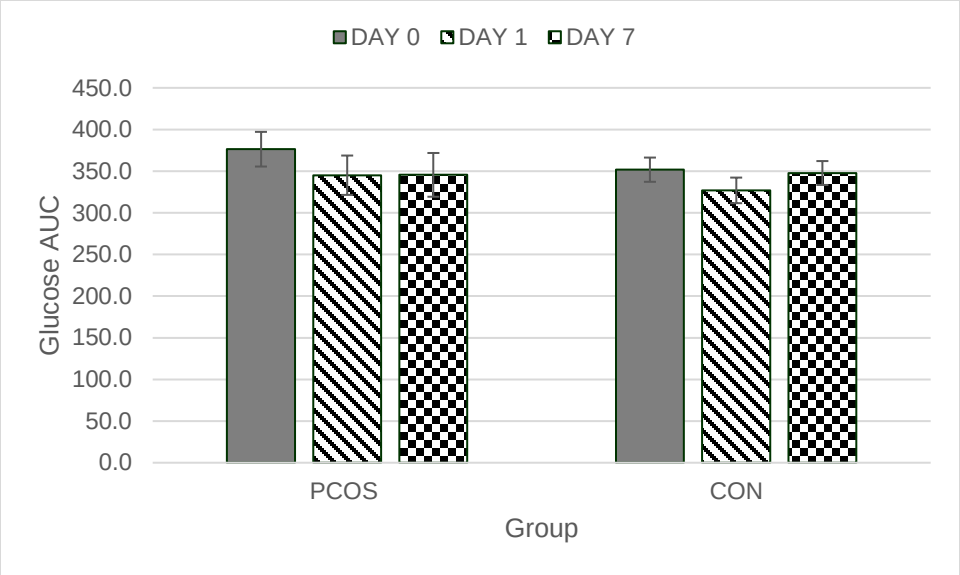

Figure 3. Mean (± S.E) Glucose AUC in PCOS and CON groups

Regardless of PCOS status, fasting glucose levels were slightly raised on Days 1 and 7 compared to Day 0 (Fig. 4). But these concentrations were statistically similar in both groups (p = .97).

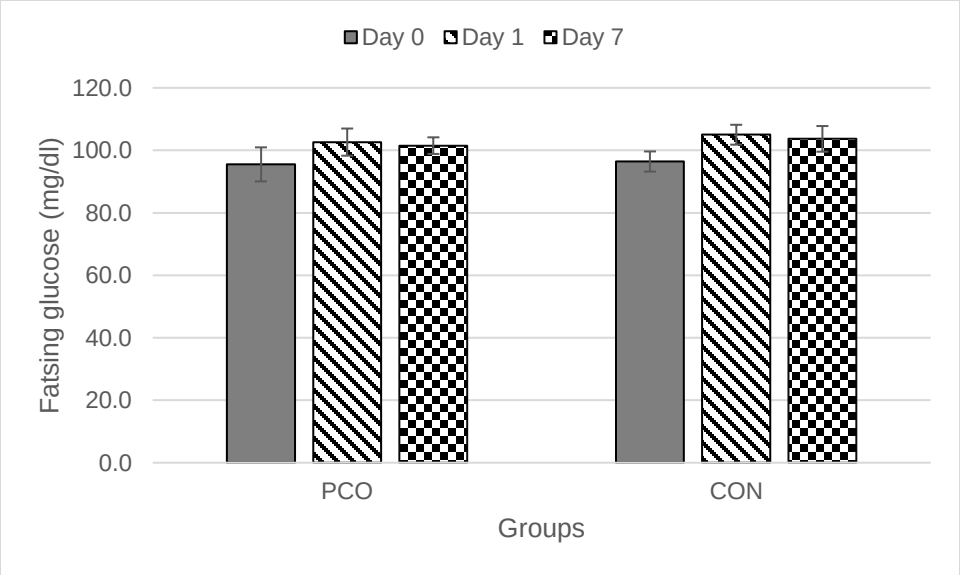

Figure 4. Fasting plasma glucose levels in PCOS and CON groups on days of OGTT

The addition of human plasma to the 3T3-L1 cells in place of FBS led to an increase in GLUT-4 expression compared to the control (PC, Fig. 5). Overall, CON-7 showed to have the highest upregulation of GLUT-4, but also had the greatest variability. Post-hoc analysis using Tukey revealed that there were significant differences between CON-0 vs. CON-7, PC vs. CON-7, PCOS-0 vs. CON-7, and PCOS-7 vs. CON-7.

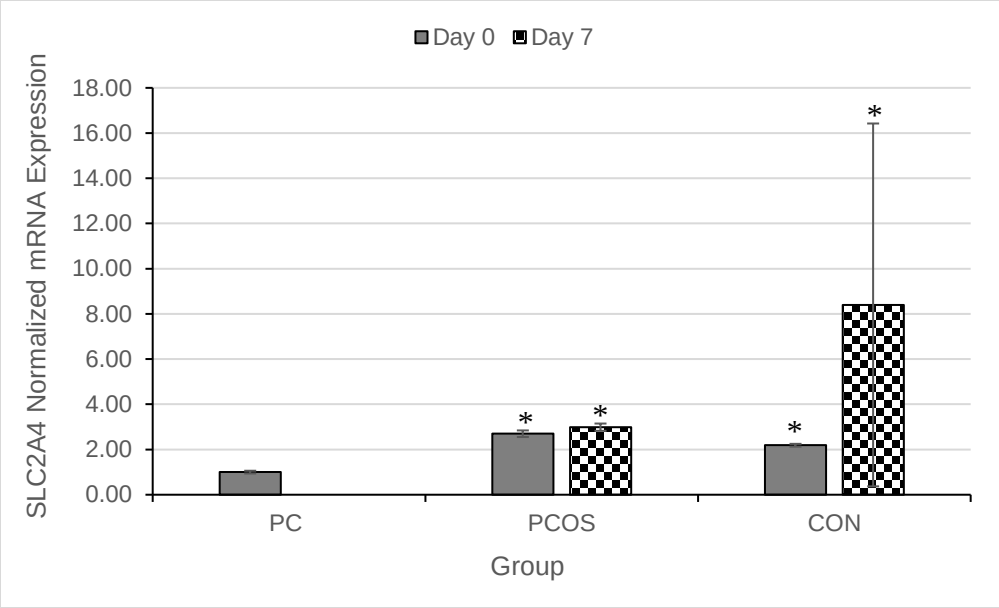

Figure 5. Changes in GLUT-4 (SLC2A4) gene expression before and after WP supplementation in control (CON), PCOS, and positive control (PC) treated 3T3-L1 cells. Data are represented as normalized mRNA expression relative to zero. Values shown as means ± SEM (* denotes $P<0.05$ from PC)

5. Discussion & Conclusions

There have been various studies examining the effect of whey protein on blood glucose levels in type 2 diabetics and PCOS populations. Mignone et al (2015) evaluated whey properties and suggested that it would make an effective tool in the management of type 2 diabetes.[10] Whey protein stimulates the secretion of insulin, especially when ingested as a preload, and can significantly reduce blood glucose levels following a meal.[10] Additionally, whey's ability to curb appetite and slow gastric emptying can contribute to whey's blood glucose lowering effect.[10] Ma and coworkers (2009) found that 55 g of whey protein ingestion prior to the consumption of a carbohydrate-heavy food or along with food stimulates insulin release, leading to lower blood glucose levels in type 2 diabetics.[9]

Jakubowicz et al (2014) tested the effect of 50 g whey protein preload in fifteen type 2 diabetics and found that blood glucose levels were significantly reduced during the 180 minutes following the meal.[11] To our knowledge, the present study is the first to demonstrate the use this particular nutritional strategy for glycemic control in women with PCOS. Our results identified a higher persistent blood glucose response in women with PCOS following a glucose tolerance test that appeared to be attenuated with whey protein preload. Although glucose AUC were similar in both groups on all three test days, glucose excursions after acute and short-term treatment were reduced in comparison to the non-whey treatment. This agrees what had been found in previous studies. The reduction in postprandial glycemia is mediated by the direct and indirect insulinogenic effect of whey protein via the gut-derived hormones[9-12].

Ezeh et al (2019) explored the connection between GLUT-1 expression, GLUT-4 expression, and insulin resistance in women with and without PCOS.[13] They reported that insulin resistance seemed to be correlated with a decrease in the expression of GLUT-4 and a lack of an increase in the expression of GLUT-1.[13] As women with PCOS tend to be more hyperinsulinemic than the general female population, it is possible that the whey effect is mediated through improved tissue responsiveness to insulin. Where GLUT-4 was upregulated in cell culture using plasma from PCOS women following whey protein ingestion, it was also upregulated in response to plasma from non-PCOS controls. The GLUT-4 evaluation was completed on plasma only from a single PCOS and a single control woman and will require additional evaluation using plasma from other PCOS and non-PCOS participants. If this pattern is repeated in additional analysis, it may indicate that if whey protein improves glucose clearance, it may do so via a non-insulin mediated process.

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, findings that are consistent with those found previously on GLUT-4 and insulin resistance studies in PCOS women. The nonsignificant difference between Day 0 and Day 7 in PCOS may be due to insulin resistance, as well as the fact that one week of whey supplementation may not provide enough time for any significant upregulation or downregulation of the GLUT-4 gene. This preliminary study may indicate that whey protein supplementation might be an effective method of improving hyperglycemia, with the ability to attenuate blood glucose elevation following a carbohydrate load. Possible further experimentation includes longer periods of whey protein ingestion to observe the glucose response and GLUT-4, as well as looking at the effects of whey protein on the changes in incretin levels in women with and without PCOS.

6. Acknowledgement

Support for this study was provided by the TWU Experiential Scholar Program, TWU Human Nutrition Research Funds, and Glanbia Nutritionals, without whom this project could not have been completed.

7. References

1. Azziz, R. et al. (2010). Polycystic ovary syndrome. *Nature Review Disease Primers, 2,* 1.
2. Insulin Resistance & Prediabetes. National Institute of Diabetes and Digestive and Kidney Diseases. https://www.niddk.nih.gov/health-information/diabetes/overview/what-is-diabetes/prediabetes-insulin-resistance. Published May 1, 2018. Accessed December 5, 2018.
3. Hooper, C. (2018). Overview of insulin signaling pathways [Poster]. Retrieved from https://www.abcam.com/pathways/overview-of-insulin-signaling-pathways
4. Type 2 diabetes. American Diabetes Association. http://www.diabetes.org/diabetes-basics/type-2/. Accessed December 5, 2018.
5. Saltiel, A. R., & Kahn, C. R. (2001). Insulin signaling and the regulation of glucose and lipid metabolism. *Nature*, *414*, 799-806.
6. Huang, S., & Czech, M. P. (2007). The GLUT4 Glucose Transporter. *Cell Metabolism,5*(4), 237-252. doi:10.1016/j.cmet.2007.03.006
7. Baptiste, C. G., Battista, M. C., Trottier, A., & Baillargeon, J. P. (2010). Insulin and hyperandrogenism in women with polycystic ovary syndrome. *The Journal of steroid biochemistry and molecular biology*, *122*(1-3), 42–52. doi:10.1016/j.jsbmb.2009.12.010
8. Spanakis, E. K., & Golden, S. H. (2013). Race/ethnic difference in diabetes and diabetic complications. *Current diabetes reports*, *13*(6), 814–823. doi:10.1007/s11892-013-0421-9
9. Ma J, Stevens JE, Cukier K, et al. Effects of a Protein Preload on Gastric Emptying, Glycemia, and Gut Hormones After a Carbohydrate Meal in Diet-Controlled Type 2 Diabetes. *Diabetes Care*. 2009;32(9):1600-1602. doi:10.2337/dc09-0723.
10. Mignone LE. Whey protein: The "whey" forward for treatment of type 2 diabetes? *World Journal of Diabetes*. 2015;6(14):1274. doi:10.4239/wjd.v6.i14.1274.
11. Jakubowicz D, Froy O, Ahrén B, et al. Incretin, insulinotropic and glucose-lowering effects of whey protein pre-load in type 2 diabetes: a randomised clinical trial. *Diabetologia*, *57*(9), 1807-1811. doi:10.1007/s00125-014-3305-x.
12. Stevenson, E. J., & Allerton, D. M. (2018). The role of whey protein in postprandial glycaemic control. *Proc Nutr Soc*., *77*(1), 42-51. doi: 10.1017/S0029665117002002.
13. Ezeh U, Chen IY-D, Chen Y-H, Azziz R. Adipocyte expression of glucose transporter 1 and 4 in PCOS: Relationship to insulin-mediated and non-insulin-mediated whole-body glucose uptake. *Clinical Endocrinology*. 2019;90(4):542-552. doi:10.1111/cen.13931.