2017

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**PURPOSE**. The aim of this study was to investigate the effect of the addition of whey, soy, milk, or pea protein to fruit-based smoothies on postprandial blood glucose levels.

**METHOD**. Eight subjects (1 male, 7 female, age 22.6 ±1.6 years, BMI 23.3 ± 2.5 kg/m$^2$) participated in a randomized controlled study. Subjects consumed a 625 mL (2.5 cups) smoothie that contained 30 grams of either whey, soy, milk, or pea protein. A smoothie with no added protein was used as a control. All subjects were given the same type of smoothie on each test day. Finger sticks were used to measure blood glucose levels at baseline, 50 minutes, and 120 minutes after consumption of the smoothies.

**RESULTS**. There was no difference in fasting blood glucose among subjects prior to drinking the smoothies ($p = .92$). The rise in blood glucose was significantly less at 50 minutes after consumption of the smoothies containing soy and whey protein in comparison to the control smoothie ($p<.05$), while at 120 minutes the soy and control were significantly lower than pea protein ($p<.05$). The blood glucose levels from the control smoothie at 50 to 120 minutes after consumption decreased significantly in comparison to all of the proteins additives.

**CONCLUSIONS**. The addition of protein to smoothies has an effect on blood glucose response. Our study found that smoothies containing soy and whey proteins produced a lower glucose response from baseline to 50 minutes (96.4 mg/dL and 101.4 mg/dL, respectively) when compared to smoothies containing protein extracts from pea, milk, or the control (103.25 mg/dL, 103.3 mg/dL, and 118.0 mg/dL, respectively).

With busy lifestyles and the challenge of having regular wholesome breakfasts, smoothies are becoming a popular option for meal replacements. There is also a trend of adding supplementary nutrients, such as various forms of protein, in order to make the smoothies more nutrient-dense, however, the addition of these supplementary nutrients can cause different magnitudes in the rise of postprandial blood glucose, as was seen in a study that evaluated cod protein versus milk and soy protein.[1] A high postprandial blood glucose spike is significant because it can cause detrimental effects to the body that are more pronounced than persistent hyperglycemia due to the lack of adaptation to high amounts of glucose metabolites in the cell.[2] Previous studies demonstrated an increased apoptotic cell rate, activation of protein kinase C, and increased reactive oxygen species (ROS) levels with temporary hyperglycemia, which would induce oxidative stress,[3-5] and could lead to cardiovascular disease, especially atherosclerosis.[6-10] An *in vivo* study also showed that a temporary rise of blood glucose after a meal can lead to reversible monocyte adhesion to endothelial cells in rats that were not insulin resistant.[2]

Protein, one of the three major macronutrients, has numerous physiological roles. Dietary protein provides indispensable amino acids that serve as building blocks for all body parts. Protein is characteristically known to keep postprandial blood sugar levels stable by slowing gastric emptying,[11] and increasing insulin secretion.[12-15] Previous studies have shown that different forms of protein are digested at different rates and therefore can have different effects on postprandial glycemic response.[1(953)]

The purpose of this graduate student research study was to investigate whether the addition of various non-fat protein isolates to smoothies would have an effect on blood glucose response. We hypothesized that the blood glucose level would not reach as high an apex when consuming smoothies containing protein isolates regardless of the type of protein as compared to smoothies without any additional protein source.

## Methods

*Subjects*

Eight healthy non-smoking volunteers, aged 20 to 45 years with normal body mass index (BMI; in $kg/m^2$ 18.5 to 24.9) and normal fasting blood glucose (less than 100 mg/dL) recruited from Loma Linda University.

All subjects were not receiving any drug treatments for the duration of the study and did not have history of soy or lactose malabsorption, diabetes, prediabetes, were not allergic to dairy, soy, whey, pea, banana, strawberry, blueberry, or orange juice, and were not lactose intolerant. Subjects did not have a history of issues in relation to coagulation and were not taking Warfarin. All methods and procedures were approved by the Institutional Review Board (IRB) of Loma Linda University. All subjects signed an informed consent.

The fasting blood glucose level of each subject was measured on each day of data collection. The normal level for fasting blood glucose is less than 100 mg/dL. Subjects with fasting blood glucose levels greater than 100 mg/dL were excluded.

*Questionnaires*

The questionnaire that subjects received was created by Da Eun Yang, one of the student investigators, and contained questions about sleep patterns, exercise history, stress levels and menstrual cycle for female subjects (See Appendix A). The stress level of subjects was collected using the Perceived Stress Scale (PSS).[17] The PSS is a short, one-page questionnaire and therefore, took approximately one to two minutes to complete. Scoring for the PSS ranges from 0 to 40.

The sleep patterns were collected via The Pittsburgh Sleep Quality Index (PSQI). Scoring for the PSQI ranges from 0 to 54, with the higher score indicating a less restful sleep. The body mass index (BMI) was calculated from height and weight measurements of each subject before the study began.

*Blood glucose*

Blood glucose levels were measured with an ACCU-CHEK Performa Blood Glucose Monitoring System using McKesson single-use lancets or comparable single-use lancets. Investigators inserted a single-use lancet into the lancing device, and pressed the lancing device against the subject's finger in order to obtain a blood sample. Investigators immediately touched the tip of the testing strip to the drop of blood in order to obtain the glucose reading. Investigators removed the testing strip and properly disposed of the lancets in a sharps bin.

*Smoothies*

The control smoothie contained no added protein powder and was used to assess the differences in glucose levels when compared to the smoothies that contained added protein powder. The recipe of the smoothies was fruit-based with added protein isolates (See Appendix B). Four different protein powders were used in the study: Virgin unflavored whey protein isolate, nonfat dairy, unflavored micellar casein protein MILK PROTEIN SMOOTH, Naked pea protein isolate, and GNC SuperFoods unflavored soy protein isolate (See Appendix C).

*Procedures*

The research design was a single-blinded, within-subject trial. Subjects received one type of smoothie on each test day and all subjects were blinded in regards to the type of protein powder in the smoothie to reduce confounding factors.

Informed consent was collected on the day of recruitment. Subjects also received a questionnaire pertaining to their sleep history, pattern, exercise intensity, current stress level, and information regarding menstrual cycle for female subjects (see Appendix A). These factors are known to have an effect on hormonal changes and basal metabolic rate (BMR), and thus interfere with the individual glycemic response.

All smoothies were pre-made and frozen before the first test day. The smoothie base, before adding proteins, was first made in small batches due to the limited volume a blender can hold. The smoothies were then combined in a large container to create a homogeneous mixture. The final volume of the smoothie base was accurately measured to 78.125 L, which was then equally divided into 125 container bags containing 625.0 mL. All smoothies were then frozen. On each test day, 25 bags of the smoothie were taken out of the freezer and defrosted under running water. The defrosted smoothies were either re-blended without any additional ingredients, or were re-blended with 30.0 g of one type of protein isolate powder: nonfat milk, pea, whey, or soy.

The smoothies were provided as breakfasts on five different occasions, twice for two weeks and once for one week of the trial. The subjects were instructed to eat normally the night before, to avoid eating and drinking fluids except water 10 hours before the start of the test, and to not use sweetened toothpaste (such as those containing saccharin and/or xylitol) when brushing their teeth. In addition, they were also instructed to avoid alcohol, caffeinated drinks, excessive physical activity, and food rich in dietary fibers 24 hours before each test.

Subjects were split into four groups, given their smoothies, and were finger pricked within 5-minutes of the previous test subject. The subjects were given 15 minutes to completely consume their smoothie, then timing began. Investigators wore new gloves each time a finger stick was performed. Finger sticks were done 50 minutes and 120 minutes immediately after subjects ingested the entire 625 mL smoothie.

*Data Analysis*

A data collection table was made to organize measurements from each subject. Statistical analysis was conducted using SPSS version 24 (SPSS, Inc., Chicago, IL, USA). Means and standard deviations of plasma glucose values from each of the smoothies were calculated and differences in plasma glucose among the five smoothies were compared using two-way repeated measures ANOVA. Statistical significance was considered at $p<.05$.

**Results**

Eight subjects were studied to test blood glucose response after drinking smoothies containing whey, soy, milk, pea protein, or control. Of the eight subjects, seven of them were female and one of them was a male. Prior to smoothie consumption, the mean (SD) age was 22.6 (1.6) years, BMI was 23.3(2.5) $kg/m^2$, stress level according to the Perceived Stress scale was 14(4.9), sleep quality was 4.1(2.0) according to the Pittsburg Sleep Quality Index, and the level of routine exercise was of moderate intensity.

Interaction between the two variables in this experiment (protein types and blood glucose time points) was significant ($p<.001$). Due to the significant interaction between the two variables, blood glucose response after consuming different types of smoothies required further analysis.

*Comparison of Blood Glucose Response versus Time*

Prior to smoothie consumption, fasting blood glucose among subjects did not differ significantly ($p = .92$). Blood glucose differences at 50 minutes after consumption of smoothies that contained whey and soy protein were significantly different from the control ($p<.05$). Blood glucose levels at 120 minutes after consumption of smoothies with soy protein or control were both significantly different from smoothies containing pea protein ($p<.05$). See Figure 1 and Table 1.

The rise in blood glucose from baseline to 50 minutes after consumption of the control smoothie was significantly higher than the rise in glucose observed when subjects consumed smoothies with added soy and pea protein. Blood glucose levels did not rise significantly from consuming smoothies containing whey and milk proteins in comparison to control. Blood glucose levels from 50 minutes to 120 minutes after consumption significantly decreased when subjects consumed the control smoothie as compared to smoothies with pea, soy, whey, and milk protein. See Table 2.

*Comparison of Blood Glucose Response versus Type of Smoothie*

Blood glucose at 50 minutes after consumption of the control smoothie was significantly different from both baseline levels and those observed at 120 minutes ($p<.05$). In addition, blood glucose after consumption of smoothies with pea protein was significantly different at baseline than at 120 minutes ($p<.05$). There was a larger variance between baseline and 50 minutes after consumption of smoothies with pea protein; therefore, leading to no significant difference between baseline and 50 minutes after consumption of the smoothie. There was a significant difference in blood glucose between baseline and 50 minutes, and baseline and 120 minutes after consumption of smoothies with whey protein ($p<.05$). See Figure 2 and Table 3.

The blood glucose levels were significantly different in soy and whey protein smoothies when compared to the control smoothie at 50 minutes. In addition, the blood glucose levels of pea protein smoothie at 120 minutes as compared to 50 minutes were significantly different($p<.05$).

## Discussion

The aim of this study was to compare the effects of different protein powders in smoothies on blood glucose response. The effectiveness of each protein was compared against a control smoothie that contained no added protein. The postprandial response of glucose rise (from baseline to 50 minutes) in whey, soy, milk, and pea protein did not significantly differ amongst each other. Additionally, the rise in blood glucose was significantly less at 50 minutes after consumption of smoothies containing soy and whey protein in comparison to the control smoothie. Initially, we hypothesized that the blood glucose level would not reach as high an apex when consuming smoothies containing protein isolates regardless of the type of protein, as compared to smoothies without any additional protein source. At the conclusion of our study, our results showed that only soy and whey proteins were consistent with our hypothesis.

Protein is effective in producing a less elevated glucose response due to its ability to stimulate insulin secretion. Post-Skagegård et al demonstrated that the intestines increase secretion of incretin hormone in the presence of dietary proteins. [1] They measured a rise in incretin hormones, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1), which induced an increased insulin response. Furthermore, postprandial blood glucose was lowered in response to amino acids (AA), specifically branched chain amino acids (BCAA). [13] The BCAAs, leucine, isoleucine, valine, lysine, and threonine in particular, act as potent promoters of insulin secretion and were shown to increase the insulin response more than other AAs.[1] Protein sources with higher BCAA content were shown to stimulate a higher pancreatic response by secretion of more insulin. This increase in insulin secretion is responsible for maintaining a more controlled glycemic response and allows for a more rapid glucose uptake, which brings blood glucose levels back down to baseline levels at a faster rate and prevents blood vessel damage from prolonged high blood glucose.

Whey protein is insulinogenic because it contains high concentrations of leucine, and is therefore considered to be one of the most rapidly digested proteins (absorption rate of 8-10 g/hr).[13, 16] In addition, whey protein stimulates glucagon-like peptide 1 (GLP-1) in a mouse model, which increases incretin while also inhibiting dipeptidyl peptidase-4 inhibitor (DPP-IV) an inhibitior of insulin release. Therefore, with ingestion of whey protein, there is continued incretin release, which results in an extended time period of insulin levels in the blood. [13]

Soy protein (valine 3.9%, leucine 7.5%, and isoleucine 3.7% of total dry product) also has higher BCAA compared to pea protein (valine 3.9%, leucine 7.3%, and isoleucine 3.4% of total dry product) but not as high as whey (valine 5.1%, leucine 9.8%, and isoleucine 5.7% of total dry product) resulting in a lower insulin response in comparison, [12] however, according to our study, soy protein showed the lowest insulin response.

The small subject pool could have led to our results. With a larger participation, the effects of the different proteins on glucose response might have led to more statistically significant results which may have been more representative of the population. Further studies with a larger subject pool and more finger sticks beyond the standard 50 minutes and 120 minutes might produce more statistically significant data and results. Further limitations included participant absorption rate, body composition and glucose tolerance. Using a younger aged population (age 22.6 ±1.6) the absorption rate of the subjects can be more efficient, meaning that peak glucose may occur before the standard 50-minute peak guidelines. Also, protein supplemented smoothies contained approximately 120 additional calories than the control smoothies. The extra calories in these smoothies with supplemented protein may have led to prolonged macronutrient breakdown, contributing to higher blood glucose two hours later.

.For future studies, the same experiment could be repeated using subjects with type 2 diabetes mellitus (T2DM) to determine if the blood glucose response would be less attenuated following ingestion of smoothies with added protein. In T2DM, an abundance of insulin is secreted and is bound to insulin receptors on cells, but the signal for cells to uptake glucose and inhibit gluconeogenesis is not sufficiently propagated. If it is found that the subjects' blood glucose response does not significantly differ between control and protein-supplemented smoothies, then enhanced insulin secretion due to BCAAs is indeed the likely mechanism that accounts for lowering the glycemic response in normal individuals, since individuals with T2DM have impaired insulin-signaling. The varying levels of insulin in the blood could be co-analyzed along with glucose to verify this effect, however, if individuals with T2DM also show diminished rises in blood glucose following a protein-doped smoothie, then perhaps a protein-induced increase in insulin secretion may not be the putative mechanism of lowering blood glucose, again, because insulin is underutilized in diabetics. The added protein may instead directly affect either intestinal absorption or cellular uptake of glucose, independent of insulin levels.

Furthermore, it would also be helpful to analyze the peak and drop of glucose more extensively. Collecting data at additional time points would allow researchers to identify the true peak of glucose absorption and determine if blood glucose levels might fall below baseline with the ingestion of different proteins due to a hyperinsulinemic response. Other future studies could look into restricting and standardizing the total calories of the smoothies. This could be helpful in investigating whether protein sparing would have an effect on its ability to control glucose, and whether the body would use the protein for muscle and cell function.

**Conclusion**

In conclusion, soy protein showed better glucose control followed by whey, pea, and milk proteins. Other studies have concluded that whey protein is the superior protein when it comes to improved glucose control. Our study concluded that smoothies containing soy and whey proteins produced a lower glucose response from baseline to 50 minutes (96.4 mg/dL and 101.4 mg/dL, respectively) when compared to smoothies containing protein extracts from pea and milk, or the control.

**Acknowledgements**

We would like to thank the participants who took part in our research study and express our appreciation to Loma Linda University for the use of their facilities to conduct the research study. We would also thank principal investigators, Kyndra Woosley, MS, RD and James Carter III, PhD for their assistance in reviewing our paper and their aid throughout the research process and Grenith Zimmerman, PhD for assistance in statistical analysis, Cindy Kosch, MS, RD and Mary K. Becker for their assistance in securing the facilities to conduct our research, Chef Cory Gheen for his aid in ordering our supplies, Lee Berk, DrPH, MPH, FACSM, CHES, CLS and Kristin Bruhjell for glucose analysis training, Leh Chang for loaning us the instruments for our research, and Gurinder Bains, MD, PhD for editing our IRB application.

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**Tables/Figures**

Table 1. Comparison of Blood Glucose Level (mg/dL) versus Time after Consumption of Smoothies Containing Whey, Soy, Milk, Pea Protein and Control for Eight Subjects at Baseline, 50 Minutes and 120 Minutes after Drinking Smoothies.

| **Type of protein added** | **Baseline** Mean (SD) | **50 minutes** Mean (SD) | **120 minutes** Mean (SD) |
|---|---|---|---|
| Whey | 85.1 (3.3) | 101.4 (12.0) | 92.4 (6.9) |
| Soy | 86.0 (9.4) | 96.4 (7.9) | 87.4 (6.0) |
| Milk | 87.6 (8.2) | 103.4 (17.9) | 93.8 (8.1) |
| Control | 87.0 (7.8) | 118.0 (17.3) | 87.3 (6.5) |
| Pea | 87.8 (10.2) | 103.3 (14.2) | 99.1 (7.8) |

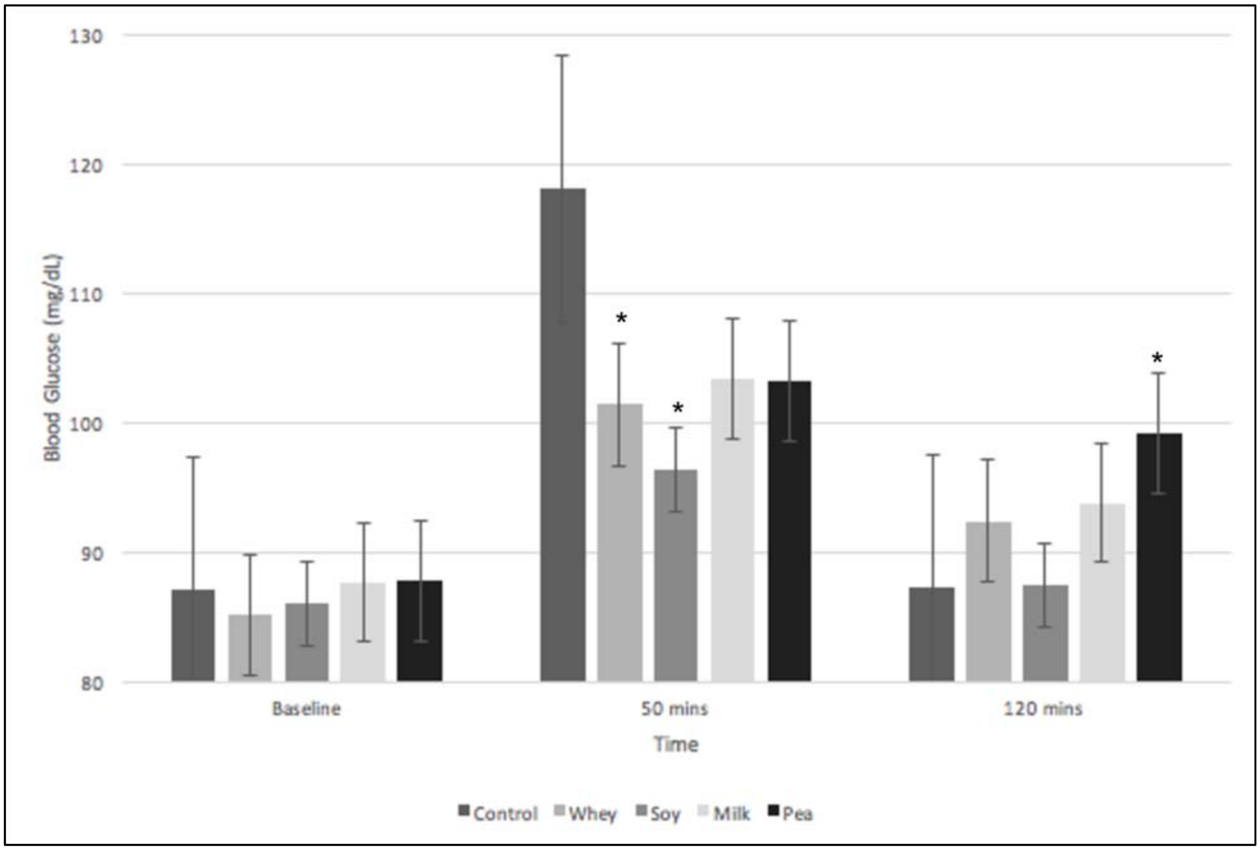

Figure 1. Comparison of blood glucose response versus time after consumption of smoothies with whey, soy, milk, pea protein and control among the eight subjects.

*Significant difference from control. ($p<0.05$)

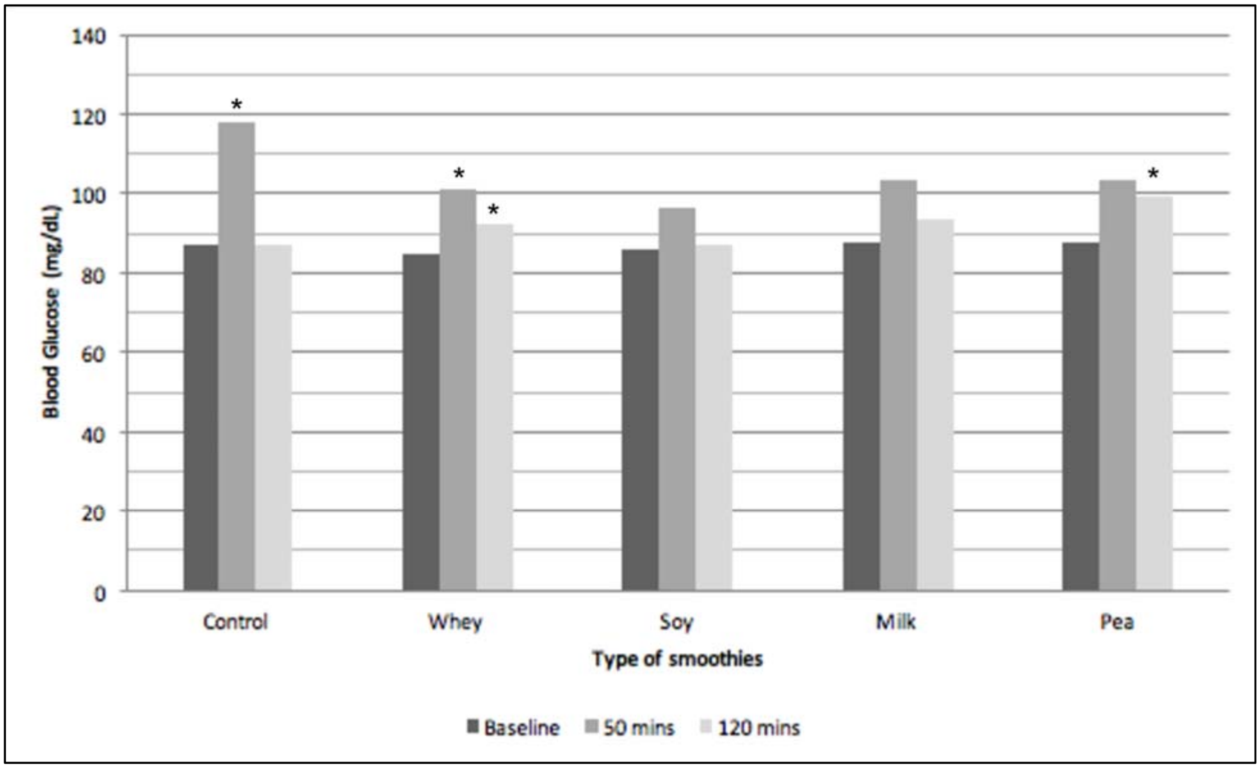


Figure 2. Comparison of blood glucose response versus type of smoothie at three time points for smoothies with whey, soy, milk, pea protein and control for the eight subjects .

*Significant difference from baseline of each protein

Table 2: Blood Glucose Changes (mg/dL) between Baseline vs. 50 Minutes, and 50 Minutes vs. 120 Minutes after Drinking Smoothies with Four Different Proteins and Control

| | Smallest changes<br>Mean (SD) | | | Biggest changes<br>Mean(SD) | |
|---|---|---|---|---|---|
| **Baseline to 50 minutes** | Soy*<br>10.4 (15.3) | Pea*<br>15.5 (15.6) | Milk<br>15.8 (18.0) | Whey<br>16.3(12.5) | Control<br>31.0 (18.0) |
| **50 minutes to 120 minutes** | Pea*<br>-4.1 (10.3) | Soy*<br>-9.0 (9.4) | Whey*<br>-9.0 (13.4) | Milk*<br>-9.6(13.3) | Control<br>-30.8 (18.2) |

*Significant difference from smoothies control.

Table 3: Blood Glucose Level (mg/dL) in Smoothies with Whey, Soy, Milk, Pea Protein and Control Among the Eight Subjects at 50 Minutes and 120 Minutes after Consumption.

| | Lowest glucose level<br>Mean (SD) | | | Largest glucose level<br>Mean (SD) | |
|---|---|---|---|---|---|
| **50 minutes** | Soy*<br>96.4 (7.9) | Whey*<br>101.4 (12.0) | Pea<br>103.25 (14.2) | Milk<br>103.3(17.8) | Control<br>118.0 (17.3) |
| **120 minute**s | Control<br>87.3 (6.5) | Soy<br>87.4 (6.0) | Whey<br>92.4 (6.9) | Milk<br>93.8 (8.1) | Pea*<br>99.1 (7.8) |

*Significant difference from control.