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RESEARCH ARTICLE

Post-exercise Glucose Response Following Whey Protein Ingestion in Healthy Young People: A Randomized Pilot Study

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Abstract:

Background:

Whey protein may have an effect directly on the muscle to affect exercise glucose response.

Objective:

The study aimed to measure post-exercise glucose recovery with supplementation and the role of DPP-IV and IL-6.

Methods:

Twenty-four participants were randomly assigned to one of three supplementation conditions (CTL: water, WPI: 31g whey protein isolate, and CHO: 32g fructose beverage; WPI and CHO beverages were isocaloric). During the Baseline Visit, participants performed an Oral Glucose Tolerance Test (OGTT) with no exercise or supplementation. On their second and third visits, participants consumed their assigned beverage then completed a maximal treadmill protocol until volitional fatigue. An OGTT was completed on the second visit, and blood samples were collected *via* venipuncture on the third visit for IL-6, Insulin and DPP-IV.

Results:

Glucose delta peak was attenuated in WPI+exercise by -45±25% and CHO+exercise by -49±21%, compared to baseline ($p<0.05$). Glucose area under the curve was only attenuated with WPI+exercise (5,993±1,013mg/dl*min), compared to baseline (10,604±4,589mg/dl*min; $p<0.05$). Insulin was elevated in the WPI+exercise (111±57pmol/L) and CHO+exercise (119±70pmol/L), compared to rest (WPI: 61±40pmol/L; CHO: 78±56pmol/L; $p<0.05$). IL-6 and DPP-IV activated T-cells (CD26+) were not different among groups. However, plasma DPP-IV was higher in WPI (8±6U/L) compared to CTL (0.7±2U/L) and CHO (0.6±4U/L; $p<0.05$).

Conclusion:

We found that a single dose of whey protein given prior to exercise results in elevated DPP-IV activity in the plasma and improved glucose response. Together these data suggest that whey protein as a supplement to exercise may be beneficial for humans trying to manage their blood sugar.

Keywords: DPP-IV, IL-6, OGTT, Whey protein isolate, Fructose, Exercise.

1. INTRODUCTION

Dipeptidyl Peptidase IV (DPP-IV) is an enzyme that cleaves the X-Pro and X-ala dipeptides from many substrates in the body including incretin hormones, many neuropeptides, and some cytokines. DPP-IV is present in almost every tissue of the body as a membrane-bound enzyme, a soluble enzyme in the blood, and as a translocatable enzyme (CD26+) on T-cells that initiates T-cell proliferation [1, 2]. Despite DPP-IV's widespread anatomy, most of the literature on this enzyme focused on DPP-IV's role in cleaving incretin hormones into their inactive form resulting in

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less insulin released from the pancreas *via* GLP-1 mediated mechanisms [1].

Considerably less is known regarding the function of DPP-IV in skeletal muscle. Work by this lab and others demonstrated that DPP-IV is also present in skeletal muscle cells [3, 4] and can be released into the interstitial space, but the function is unclear. DPP-IV can inactivate many cytokines and it is possible that muscle released DPP-IV may inactivate the muscle released cytokine, IL-6 [5]. IL-6 plays an important role in mediating muscle glucose metabolism likely through GLUT4 translocation [5]. This may be particularly true following exercise because IL-6 is released during exercise [6 - 8] and enhances glucose uptake by the muscles [5, 7]. However, excessive IL-6 release may be detrimental for health [9]; therefore, DPP-IV cleavage of IL-6 may allow reasonable balance in IL-6 release and optimal GLUT4 translocation.

Foods like whey protein and sweetened drinks may also alter the DPP-IV release from the muscle and mouth, respectively [3, 10, 11]. Considerable research suggests that pre-meal whey protein supplementation enhances post-meal glucose uptake in healthy and diabetic individuals [12 - 14]. This is generally attributed to improved gastric emptying and possibly inhibition of DPP-IV *via* whey protein [12 - 14]. In contrast, this lab demonstrated that whey protein ingestion in rats results in increased skeletal muscle DPP-IV mRNA, and applying whey protein to the media results in enhanced release of DPP-IV *via* skeletal muscle cell cultures [3].

If the effect of whey protein translates to healthy humans, we hypothesize that ingestion of whey protein before exercise will result in increased plasma DPP-IV and IL-6. This will result in improved glucose uptake following an oral glucose tolerance challenge. Whey protein will be compared to the control condition of water and an isocaloric fructose drink, which is designed to result in similar insulin changes, but not increased DPP-IV.

2. MATERIALS AND METHODS

2.1. Participants

Healthy, non-diabetic subjects (n=24) on normal diets and not currently taking medications known to affect glucose metabolism were recruited *via* the SONA system (an online participant recruitment system for recruiting students in College of Education classes), and through advertising around campus and in the community. Written informed consent was obtained from each participant prior to data collection. Participants were classified as sedentary, according to ACSM Guidelines, as completing less than 150 minutes of moderate physical activity per week [15], and individuals on special diets or supplements were excluded from the study. The flow diagram for enrollment is included in Fig. (**1**). The study is a pilot study using random assignment and a pre-test post-test design with a control group.

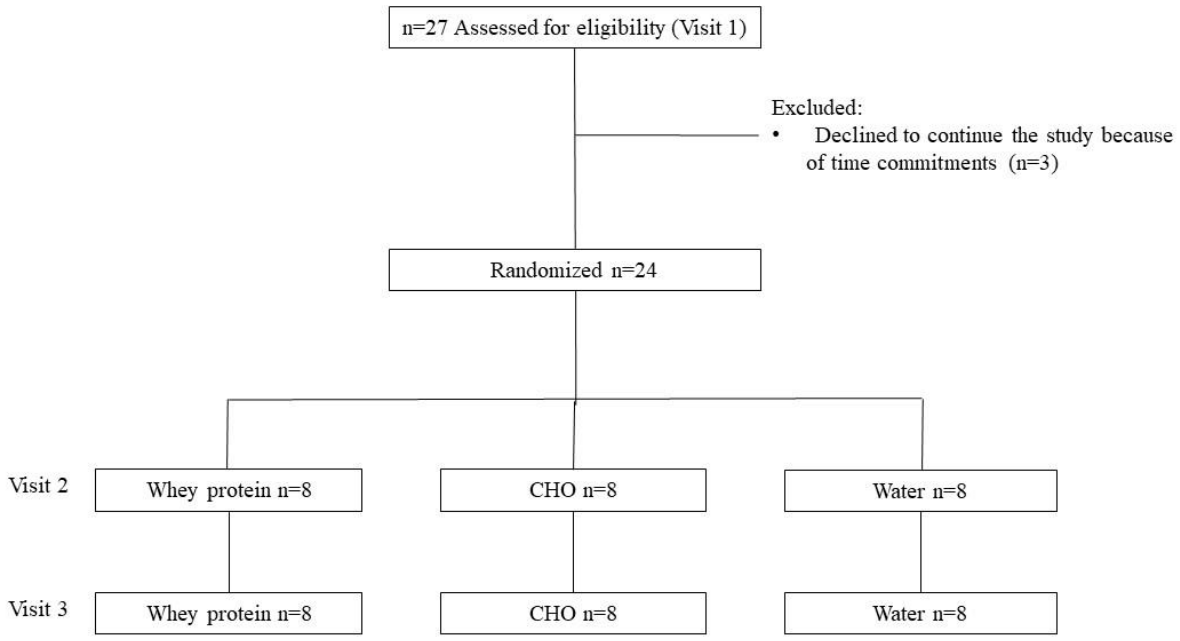

Fig. (1). Flow diagram of enrollment in the study.

The visits described below are diagramed in Fig. (**2**) and occurred in the Kinesiology Building at Auburn University, Auburn AL.

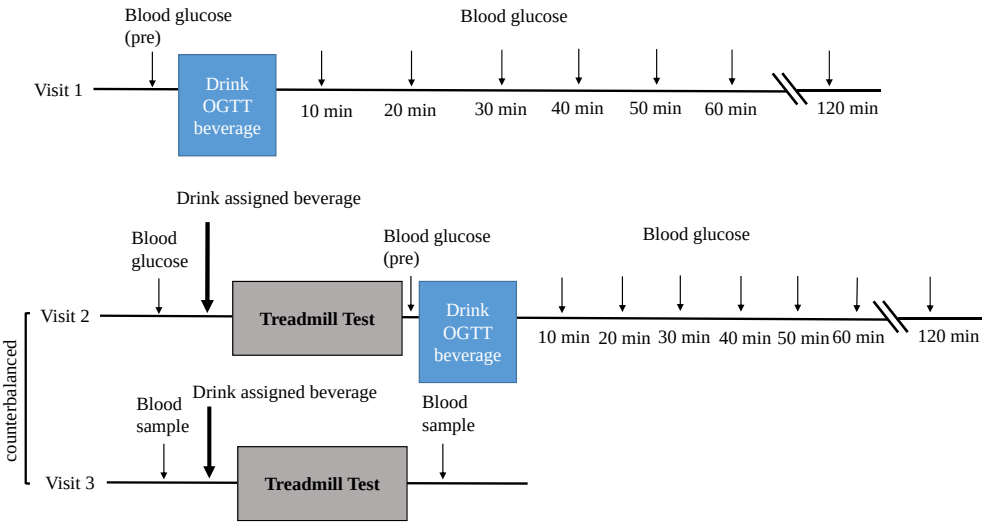

Fig. (2). Diagram of visits 1-3 with sample collection. Blood glucose was measured using an Accucheck Aviva Plus blood glucose meter. The Oral Glucose Tolerance Test (OGTT) was performed using a glucose tolerance drink (Azer Scientific). The blood sample in visit 3 was used to measure Dipeptidyl peptidase IV activity, IL-6 and for flow cytometry (CD4+ and CD26+ cells). The treadmill test was a Bruce Protocol treadmill test. On the first visit with a treadmill test, the participants exercised to volitional fatigue. For the following visit, they did a Bruce protocol treadmill test for the same amount of time as the previous visit.

2.2. Visit 1

After an overnight fast, baseline blood glucose was measured by a commercially available blood glucose monitor (Accucheck Aviva Plus meter and test strips, Roche Diagnostics, Indianapolis, IN) *via* a capillary sample from a finger. Participants completed an Oral Glucose Tolerance Test (OGTT) by drinking a commercially available glucose drink (75g of glucose per 296 ml drink; Azer Scientific, Inc., Morgantown, PA), followed by blood glucose measurements every 10 minutes until the glucose was cleared from the blood (blood glucose <140 mg/dl [16]). This test was used as a baseline measure for blood glucose uptake and to verify that participants had normal glucose tolerance (Blood glucose did not exceed 200mg/dl at any point during the test and values fell below 140mg/dl within 120 minutes [16]). Participants were excluded from the study if they failed to respond normally to the test. No participants were excluded based on this criteria.

2.3. Group Assignments

After Visit 1, the participant was stratified by gender and randomly assigned (using a random number generator) to either a control (CTL; water), a high fructose beverage (CHO; 32g fructose, NOW FOODS, Bloomingdale, IL and <4.14 ml lemon juice for flavor), or a whey protein supplementation drink (WPI; 31g whey protein, Optimum Nutrition, Aurora, IL). The CHO and WPI drinks were isocaloric (120 calories per drink). Visits 2 and 3 were scheduled at least 72 hours apart and occurred within 7 days of Visit 1.

2.4. Visit 2

Participants came to the laboratory after an overnight fast. Their baseline blood glucose was measured, and they consumed their assigned beverage. Fifteen minutes after ingestion of the beverage, participants performed a Bruce Protocol treadmill test, as stated in the ACSM Guidelines for Exercise Testing and Prescription [15], until volitional fatigue. A blood glucose measurement was taken (Pre) and then participants completed an OGTT fifteen minutes after the end of the treadmill test using the same protocol as described in visit 1. Blood glucose was measured every 10 minutes until the glucose was cleared from the blood. Blood glucose area under the curve (blood glucose-AUC) was

determined based on the readings from the OGTT using Prism data analysis software (GraphPad Software, Inc., La Jolla, CA), which utilizes the standard formula of ΔX*(Y1+Y2)/2 for each adjacent pair of points of the curve. Blood glucose delta peak was calculated as Peak blood glucose measure - Pre blood glucose measure.

2.5. Visit 3

Following an overnight fast, participants came to the lab, and venous blood samples were taken from the antecubital region (one 10ml Lithium Heparin tube for plasma analysis and one 10ml K+EDTA tube for flow cytometry analysis). They ingested their assigned beverage and waited 15 minutes before completing a time-matched Bruce Protocol Treadmill Test, such that participants ran the same time and stage as Visit 2. The second set of blood samples was collected by standard venipuncture 30 minutes post-exercise, based on previous studies demonstrating this time point was optimal for seeing changes in our variables [6, 17]. Blood collected in the Lithium Heparin tubes was centrifuged at 1000G for 10 minutes at 4°C to separate the plasma, which was stored at -80°C in ~1ml aliquots until analysis.

2.6. Flow Cytometry

The blood collected in the K+EDTA tubes was processed for flow cytometry on the same day of the sample collection. The samples were centrifuged at 400G for 20 minutes at room temperature to separate the red blood cells, Peripheral Blood Mononuclear Cells (PBMCs), and plasma. Following centrifugation, the pellet, which was resuspended in 1ml of Flow Cytometry Staining Buffer (FCSB; Thermo Fisher Scientific, Waltham, MA) and placed on ice. The CD4+ antibody (CD4 PE, BD Biosciences, San Jose, CA) and CD26+ antibody (CD26 FITC, BD Biosciences, San Jose, CA) were prepared and combined with the pellet. The sample was read in the flow cytometer, for a total of 300,000 events, and gated according to their log side scatter and forward scatter characteristics. Values are expressed as percentages of the total lymphocytes for CD4+ T-cells, CD26+ T-cells, and CD4+/CD26+ T-cells [2].

2.7. IL-6, Insulin, and DPP-IV Analysis

Plasma IL-6 and insulin were measured by ELISAs (AB46042 and AB200011, Abcam, Cambridge, MA) as per the manufacturer's instructions. Plasma DPP-IV activity was determined using a fluorometric assay developed by [18] and used in several previous studies by this lab [3, 19 - 21]. Briefly, DPP-IV activity was determined by the following equation:

$$\text{Activity (U/L)} = [(S) \times V_A \times 1000 \times C_{st})]/ [(T \times S_v) \times (F)],$$

where S is the sample fluorescence minus the sample blank fluorescence, V_A is the total volume of the well, C_{st} is the standard concentration, T is the time of incubation, S_v is the sample volume, and F is the standard fluorescence minus the standard blank fluorescence.

2.8. Statistical Analysis

All data are presented as mean ± standard deviation. Statistical significance was determined by a two way repeated measures ANOVA, where condition (supplement taken) and time (Baseline or Following Exercise) served as the factors. Where the changes in blood measures were analyzed for statistical significance, a one way ANOVA was performed. Post-hoc analysis was used to determine the significant difference between and within groups, when necessary. The sample size was determined by an a priori analysis using G-Power 3.1.5 based on the effect size of IL-6 obtained using data from Landers-Ramos *et al.* [6]. To achieve a large effect size, with a power equal to 0.95, a sample size of 18 participants was required.

3. RESULTS

3.1. Participant

Participant by group characteristics are presented in Table **1**. Of the 27 recruited and consented participants, 24 participants completed all three visits. The data presented in the results refer only to the 24 that completed all of the visits. Fasting blood glucose was not different among the groups at Visit 1 (93±10mg/dl) or Visit 2 (90±11mg/dl). The groups were not different for height, weight, BMI or age.

Table 1. Participant characteristics by group.

-	Water (CTL)	Whey Protein (WPI)	Fructose (CHO)
Number of participants	Men=3 Women=5	Men=3 Women=5	Men=6 Women=2
Height (cm)	167±8	178±8	169±8
Weight (kg)	71±8	78±13	65±13
BMI (kg/m^2)	26±4	24±3	23±3
Age (years)	23±2	21±1	23±2
Final Stage of Bruce Protocol Reached	4±1	4±1	4±1
Bruce Protocol Time Completed (minutes)	12.5±1.7	12.0±1.9	13.9±1.3

Mean ± standard deviation.

There are no significant differences among the groups for characteristics (Table **1**).

3.2. Blood Glucose Measurements

Blood Glucose delta peak was decreased with WPI+exercise and CHO+exercise, compared to their respective resting conditions (Cohen's d=0.66; p<0.05). There was no difference in blood glucose appearance in the CTL group (Fig. **3A**). When blood glucose measurements were presented as blood glucose area under the curve (blood glucose-AUC), there was a significant interaction (p<0.05) among the type of supplement taken and exercise condition. Post hoc analysis revealed that resting measurements were not different, but a significantly smaller blood glucose-AUC occurred when exercise was performed following whey protein ingestion (Fig. **3B**).

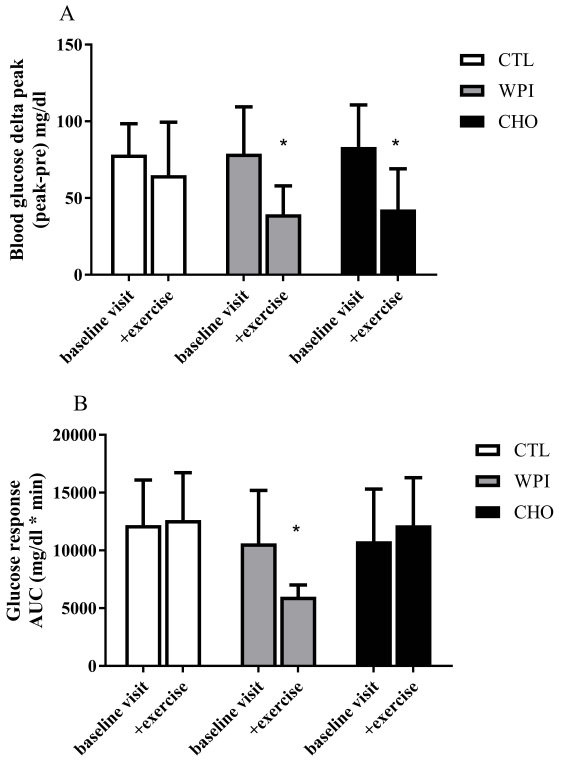

Fig. (3). The mean blood glucose delta peak for visit 1 (baseline visit) *versus* visit 2 (+exercise) (**A**). Blood glucose delta peak was calculated as the difference between peak blood glucose and the "pre" blood glucose for that visit. Blood glucose delta peak was attenuated by WPI+exercise and CHO+exercise, but not by exercise alone (CTL+exercise) (*p<0.05 different from the baseline visit). There were no differences among the groups at the baseline visit (visit 1). The mean glucose response presented as an Area Under the Curve (AUC) (**B**). When the entire glucose response curve was taken into account, WPI+exercise resulted in faster glucose response compared to the baseline visit (visit 1; p<0.05 different from the baseline visit). There was not difference with CHO+exercise or CTL+exercise compared to the respective baseline visits.

3.3. Plasma Insulin and IL-6 Measurements

Resting plasma insulin was not significantly different among groups (combined mean of three groups: 66.2±40.8 pmol/L). Plasma insulin did not change significantly in the CTL group, but it was significantly increased from rest in the WPI and CHO groups following exercise (Cohen's d=2.09; p<0.05; Fig. **4**). However, the WPI and CHO groups exhibited comparable insulin release following exercise. Resting plasma IL-6 was not significantly different among groups (combined mean of three groups: 3.98±7.55 pg/ml). Plasma IL-6 significantly increased following exercise in the CTL (rest: 1.16±0.87; post-exercise: 1.63±0.86 pg/ml) and WPI groups (rest: 7.72±12.06; post-exercise: 8.12±12.25 pg/ml) (p<0.05), but was not changed in the CHO condition (rest: 2.91±2.75; post-exercise: 2.86±2.86 pg/ml). We were unable to detect IL-6 in one participant in the CHO group at rest and post-exercise, therefore, the n=7 in the CHO group for IL-6. However, when the data was expressed as a difference from rest, there were no significant differences among the groups (Fig. **5**).

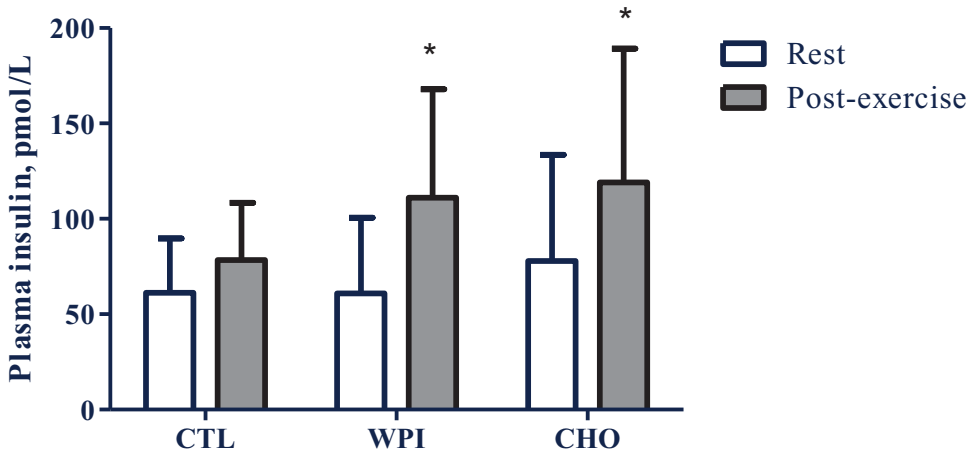

Fig. (4). The average plasma insulin before the beverage was consumed (rest) *versus* plasma insulin after exercise (post-exercise) in visit 3. Plasma insulin was elevated following exercise with the consumption of WPI and CHO (P<0.05 different from rest). However, the plasma insulin increase with WPI and CHO were not different from each other.

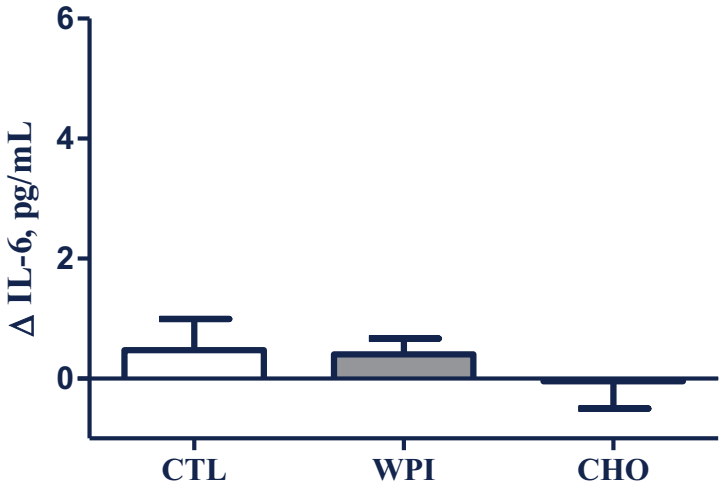

Fig. (5). The mean change in plasma IL-6 concentration from rest (pre-beverage) to post-exercise in visit 3. The change in IL-6 was not significantly different among groups.

3.4. DPP-IV Activity

Plasma DPP-IV activity at rest was not different among the groups (combined mean of three groups: 48.99±10.21U/L). The change in plasma DPP-IV activity from rest to exercise was greater in the WPI group compared

to CHO and CTL (Cohen's d=0.56; Fig. **6**). However, T-cells that expressed DPP-IV on the surface (CD26+) were not different among the conditions (CTL: -0.56±0.85; WPI: -0.52±0.81; CHO: 0.05±1.29 CD4+/CD26+ count change from rest)

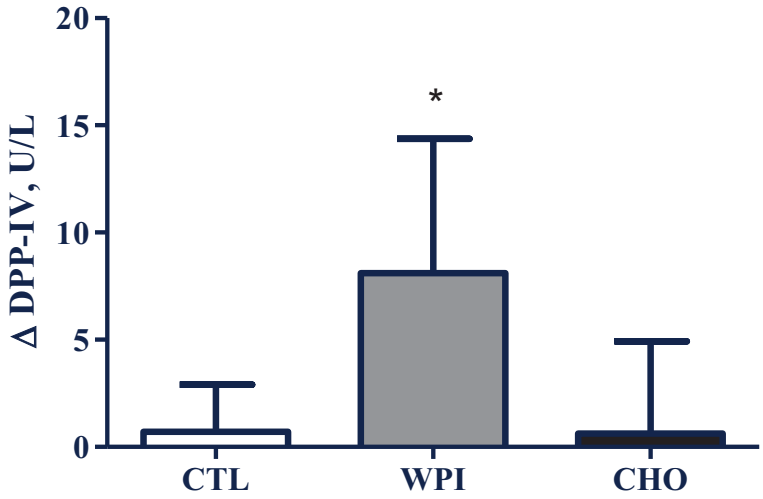

Fig. (6). The change in plasma dipeptidyl peptidase IV (DPP-IV) from rest (pre-beverage) to post-exercise in visit 3. The change in DPP-IV was greatest in the WPI condition (*p<0.05 different from CTL and CHO). DPP-IV did not increase significantly in the CTL or the CHO conditions.

We also looked at the relationship between plasma DPP-IV activity and plasma IL-6 concentration in all conditions. We found no relationship between DPP-IV and IL-6 when all conditions were included (R^2=0.01 to 0.04).

4. DISCUSSION

We found that glucose response following OGTT was improved following whey protein ingestion, but not with CHO or the control conditions. This improvement in glucose uptake with whey protein was accompanied by increased plasma DPP-IV, but no change in IL-6. These findings suggest that whey protein may act on the skeletal muscle to improve glucose response possibly *via* DPP-IV.

4.1. Blood Glucose Measurements

Blood glucose appearance was lower in the whey protein (WPI) and the fructose (CHO) condition with exercise following an OGTT challenge. The addition of exercise in the water ingestion (CTL) group did not change the blood glucose delta peak compared to the baseline visit where no exercise was performed. This is in contrast to some studies that showed changes in insulin sensitivity [22 - 24] or lower glucose measurements [22, 25 - 27] following a single exercise bout, but one study found no change in either insulin or glucose [28]. The difference between these studies and the current study is the length of time of the exercise bout. In all of the studies mentioned the shortest exercise bout was 40 minutes [23] and the longest was 83 minutes [27]. In the current study, our participants performed a maximal exercise test that had an exercise intensity equivalent to previous studies, but the length of time exercised was only 11-15minutes. This may be too short of an exercise bout to see changes in insulin and glucose. We found that insulin was elevated in the WPI and the CHO conditions compared to the pre-drink baseline. There was no change in insulin with water ingestion (CTL) and exercise.

We also investigated glucose response and found that the rate of glucose response was increased in the WPI with exercise condition, compared to baseline with no supplement or exercise. No improvements were seen in glucose response in the CHO and CTL compared to Baseline. Given the systemic nature of our measurements, it is not possible to conclude that the improved response of glucose seen is due solely to skeletal muscle, as other organs such as the liver are capable of taking up glucose. However, it can be suggested that the exercise increased blood flow to the muscle, increasing the odds of skeletal muscle glucose response.

Previous research supports the idea that whey protein can improve post-meal blood glucose and insulin in humans [12]. Akhavan *et al.* [12] speculated this was due to faster gastric emptying induced by whey protein. This is a possible

explanation for our findings since Trahair *et al.* [29] showed a relationship between the change in blood glucose and gastric emptying in healthy people. Numerous studies have demonstrated that whey protein and other milk proteins reduce appetite and possibly increase some incretin hormones [13], which include GLP-1 that signals for the release of insulin. It is possible that this mechanism plays a role in the increased uptake of glucose from the bloodstream.

Studies looking at the effects of chronic whey protein supplementation on glucose tolerance have yielded contradictory results. Pezeshki *et al.* [30] used obese rats and saw that 8 weeks of whey protein isolate (290g/week, 29% of total diet) resulted in improved glucose tolerance and reduced weight gain, whereas, Betik and colleagues [31] reported no effect of weight gain, glucose tolerance, or insulin sensitivity in obese rats supplemented with whey protein isolate (8% of total energy) for 10 weeks. A similar result was found in obese humans supplemented with whey protein (20, 40 or 60g/day) for 36 weeks [32]. However, the participants in our study were healthy and only given an acute supplementation.

There have also been inconclusive findings when whey protein is combined with an exercise bout. In healthy humans receiving whey protein before and during a 120 minute cycling challenge, blood glucose was reduced compared to those who consumed alanine and placebo [17]. Qin *et al.* [33] provided participants with a whey protein carbohydrate combination drink during a long exercise session. Following the exercise bout, they found a smaller AUC for blood glucose with the whey protein condition, but it was not significant. In the current study, a short bout of exercise preceded by a whey protein beverage improved glucose delta peak and glucose response. This was, in part, mediated by increased insulin.

4.2. Plasma IL-6 and DPP-IV

Plasma IL-6, DPP-IV, and T-cell associated DPP-IV (CD26+ T-cells) were measured in the plasma before and after the supplementation and exercise bout during the third visit. Some participants in the whey protein group had higher IL-6 levels at rest and during exercise, but the change from rest to post-exercise IL-6 values was not different among the groups. This is in support of studies that did not see an increase in IL-6 following moderate to vigorous exercise [34 - 36]. Philippe *et al.* [34] did report significant increases in IL-6 with eccentric exercise; however, eccentric exercises are known to cause muscle damage, which may explain the exercise induced IL-6 release. In contrast to our findings, Landers-Ramos *et al.* [6] reported large changes in IL-6 with 30 minutes of treadmill exercise at a moderate to vigorous exercise intensity.

Studies have shown conflicting results on the effect of treadmill running with supplementation on IL-6. One study found that regardless of carbohydrate ingestion, plasma IL-6 was not significantly increased following a bout of running, but skeletal muscle IL-6 mRNA was significantly increased [36]. Schroer *et al.* [17] showed that IL-6 was lower with whey protein, compared to placebo during a 60min bout of intense cycling. Another study investigating whey protein's effects on muscle damage showed that while markers of muscle damage were decreased with multiple supplementations throughout the 60 minute exercise bout, it was not able to significantly attenuate the increase of IL-6 [37]. It is possible that longer exercise times and the timing of supplementation result in different interactions between whey protein and IL-6.

Plasma DPP-IV was increased after exercise in the whey protein condition, but not in the water or CHO ingestion conditions. GLP-1 is also a target for DPP-IV and increases with whey protein feeding in some studies [30, 38, 39], but not others [40, 41]. GLP-1 was not measured in this study, but it is possible that plasma DPP-IV did increase because of an increase in GLP-1. Evidence against this possibility is that we performed a whey protein feeding study in humans (same dose as the current study) without exercise and saw no change in plasma DPP-IV [3]. This is also supported by other studies that feeding or an oral glucose tolerance test alone does not increase plasma DPP-IV [42, 43].

Moreover, several studies have shown that plasma DPP-IV levels are very stable, as it is difficult to change the systemic levels of DPP-IV. Neither a single bout of intense running on the treadmill nor a single bout of intense resistance training was able to change plasma DPP-IV [3].

Previous work also implied that whey protein may act as a DPP-IV inhibitor [44, 45]; however, these studies were performed in test tubes and plates. There is some evidence in rats that the whey protein inhibition of DPP-IV may be compartmentalized, such that whey protein acts as a DPP-IV inhibitor in the gut, but does not inhibit DPP-IV in the plasma [41]. This lab has not performed experiments in the gut, but has performed a series of experiments in animals and cell culture suggesting that whey protein promotes DPP-IV release from the membrane in skeletal muscle [3].

We have also previously performed single bout intense running on the treadmill and single bout intense resistance

training and seen no increase in plasma DPP-IV [3]. Therefore, it appears that in order to see an increase in plasma DPP-IV, whey protein feeding must be combined with exercise. While we cannot ensure that the increase in plasma DPP-IV is exclusively from the muscle, evidence from this study combined with previous research increases the possibility that we are measuring DPP-IV that is, in part, released from the muscle.

Because the change seen in IL-6 was not significantly different among the three groups, the data does not support the idea that DPP-IV is directly attenuating the IL-6 concentrations. However, it is possible that it could play a small role. The IL-6 values measured in this study were at the bottom end of the assay's detection limits and further investigations into this mechanism will have to cause a greater increase in plasma IL-6 to achieve a more definitive answer. Previous work in spontaneously hypertensive rats suggested that there is a connection between DPP-IV and GLUT4 receptor expression. They found that inhibition of DPP-IV increased GLUT4 expression in the heart and skeletal muscle [46]. This finding is not consistent with our current work, but suggests that there may be a complex relationship between DPP-IV and glucose uptake.

We also measured CD26+ T-cells to query if T-cell activation via fructose [47 - 49] or inhibition *via* whey protein [50] might affect the results. We found no significant differences in the ratio of CD4+ cells (control) *versus* the CD26+ cells in any condition. This suggests that T-cell DPP-IV does not contribute significantly to the effect seen with whey protein.

CONCLUSION

In conclusion, we found that a single dose of whey protein given prior to exercise results in elevated DPP-IV activity in the plasma and improved glucose response. The IL-6 values were very low with this intensity and duration of exercise, such that we were not able to conclude that DPP-IV is a modulator of IL-6. CD26+ (DPP-IV expressing T-cells) were not elevated with any of the feeding conditions giving evidence that inflammatory induced DPP-IV was not a contributing factor in our results. Together these data suggest that whey protein as a supplement to exercise may be beneficial for humans trying to manage their blood sugar. Future studies should assess if longer-term whey protein use combined with exercise has the same or a potentiating effect on blood glucose control.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All parts of this study were reviewed and approved by the Institutional Review Board at Auburn University prior to the beginning of the study (Protocol # 15-304 MR 1507).

HUMAN AND ANIMAL RIGHTS

No animals were involved in this study. The reported experiments involving humans conformed to the standards set by the latest revision of the Declaration of Helsinki.

CONSENT FOR PUBLICATION

No identifiable data is used in this report. However, written informed consent was obtained from each participant prior to data collection.

CONFLICTS OF INTEREST

The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The authors have no conflicts of interest, financial or otherwise. This study was funded by an Auburn University, College of Education Seed Grant.

ACKNOWLEDGEMENTS

Declared none.

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