BRIDGING SPORTS NUTRITION & DIABETES CARE: ASSESSING THE ROLE OF PROTEIN INTAKE AND TIMING ON GLYCEMIA DURING AND FOLLOWING PHYSICAL ACTIVITY AMONG ADOLESCENTS AND ADULTS WITH TYPE 1 DIABETES

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Nutrition in the Gillings School of Global Public Health.

Chapel Hill
2023

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ABSTRACT

Franklin R. Muntis: Bridging Sports Nutrition & Diabetes Care: Assessing the Role of Protein Intake and Timing on Glycemia During and Following Physical Activity Among Adolescents and Adults with Type 1 Diabetes
(Under the direction of Elizabeth J. Mayer-Davis)

For people with type 1 diabetes (T1D), the risk of experiencing hypoglycemia is elevated during and up to 24 hours following physical activity, the fear of which is a leading barrier to physical activity among this population. Increasing dietary protein intake, as recommended by sports nutrition guidelines, may promote a mild, prolonged hyperglycemic effect which may mitigate the risk of exercise-related hypoglycemia. Therefore, the goal of this dissertation was to evaluate the role of protein intake and timing on exercise-related glycemia among adolescents and adults with T1D.

Data from the Flexible Lifestyles Empowering Change (FLEX) study were used to evaluate the role of pre-exercise, post-exercise, and daily protein intake on glycemia during and following bouts of moderate-to-vigorous physical activity (MVPA) among adolescents with T1D (n=112) utilizing mixed effects regression modeling. Dietary intake and MVPA were quantified using 24-hour dietary recalls and previous day physical activity records, respectively. Percent time in recommended glucose range (TIR, 70-180mg/dL), percent time below range (TBR, <70mg/dL) and percent time above range (TAR, >180mg/dL) during MVPA bouts as well as from bout cessation until the following morning were calculated from continuous glucose monitoring (CGM) data. Additional secondary analyses were conducted using data from an exercise pilot study to evaluate the role of post-exercise protein intake on post-exercise glycemia

among adults with T1D following supervised bouts of moderate-intensity continuous training (MICT) or high-intensity interval training (HIIT) performed following an overnight fast. Dietary intake was quantified using 3-day food records and TIR, TBR and TAR were calculated using CGM data.

Protein intakes of at least 10g or 0.125g/kg consumed within 4 hours prior to MVPA were associated with reduced TBR among adolescents with T1D. Additionally, daily protein intakes within sports nutrition guidelines (1.2-2.0g/kg/day) were associated with reduced TAR and increased TIR following MVPA, with stronger associations identified for adolescents with overweight or obesity, those using multiple daily insulin injections, and females. Furthermore, there was a trend ($p=0.05$) towards reduced TBR following MICT, but not HIIT among adults with T1D. Following sports nutrition guidelines for protein intake may improve the glycemic response to exercise for people living with T1D.

To my amazing wife, my best friend and adventure partner, Shelby Muntis

ACKNOWLEDGEMENTS

First, I would like to share my heartfelt thanks to all the adolescents and adults who participated in the Flexible Lifestyles Empowering Change (FLEX) and the Metabolic, Hormonal, and Physiological Characterization of Isoenergetic High Intensity Interval Training and Moderate Intensity Continuous Training in Adults with Type 1 Diabetes (HI1T) studies. The type 1 diabetes community is extraordinary in their commitment to research to help improve the lives of others in their community. Thank you for generously sharing your time, sweat and life experience with us to make this work possible.

I would also like to thank the National Institutes of Health who funded the first two years of my doctoral studies through a National Research Service Award (Award Number: 2-T32-DK007686) and provided funding that made the FLEX study possible (Award Number: UC4DK101132). I would also like to thank the North Carolina Diabetes Research Center for their support in providing funding which made the HI1T study possible.

I am also extremely thankful to have had the opportunity to "stand on the shoulders of giants" during my time at UNC with my outstanding dissertation committee consisting of Dr. Elizabeth Mayer-Davis, Dr. S. Raza Shaikh, Dr. Abbie Smith-Ryan, Dr. Kelly Evenson, and Dr. Jamie Crandell. They are all outstanding researchers, teachers, and mentors and I am grateful for all their time, training and support that has been invaluable to my development as a scientist. I would especially like to thank Dr. Elizabeth Mayer-Davis who over the past 6 years has been my advisor, role model, advocate, friend, and the greatest mentor I've ever had. No matter how busy

her schedule, Beth always found time to help me work through challenges, review and develop my writing and presentation skills, and to help me find support when a global pandemic or life event made things feel overwhelming. I am immensely grateful for all the time, patience and support she has shared with me to help me develop into the dietitian, scientist and person I am today.

Lastly, I am grateful to my friends and family who have been by my side through all the joys and struggles we have faced over the years. I can't express just how grateful I am for all their humor, advice, and friendship. Most of all, to my amazing wife, my best friend and adventure partner, Shelby Muntis, whose constant belief in me, support, friendship, and unconditional love made this work possible and has made me the man I am today. You are my favorite part of life and I love you more than I did yesterday and somehow less than I will tomorrow. Always with all my heart and soul. And to our baby girl on the way, our little nugget, who has been the greatest motivator, you are your daddy's pride and joy. I love you fiercely and always will.

PREFACE

Chapters 3, 4, and 5 represent three original articles. Chapters 3 & 5 are under review in academic journals and Chapter 4 has been published in the journal *Nutrients* (PMID:37111199).[1] This work was done in collaboration with other researchers, students, and clinicians who have provided feedback throughout the analysis and writing process.

Chapter 3 contains material produced with feedback from the following writing group: Abbie Smith-Ryan*, Jamie Crandell*, Kelly Evenson*, David M. Maahs, Michael Seid, Saame R. Shaikh*, and Elizbeth J. Mayer-Davis*.

Chapter 4 contains material produced with feedback from the following writing group: Abbie Smith-Ryan*, Jamie Crandell*, Kelly Evenson*, David M. Maahs, Michael Seid, Saame R. Shaikh*, and Elizbeth J. Mayer-Davis*.

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LIST OF ABBREVIATIONS

1RM	One-Repetition Maximum
ADA	American Diabetes Association
APL	Applied Physiology Lab
ASA-24	Automated Self-Administered Dietary Assessment Tool
BMI	Body Mass Index
CGM	Continuous Glucose Monitoring
CI	Confidence Interval
CSII	Continuous Subcutaneous Insulin Infusion
DCCT	Diabetes Control and Complications Trial
DHQ-III	Diet History Questionnaire III
DXA	Dual-Energy X-Ray Absorptiometry
EDIC	Epidemiology of Diabetes Interventions and Complications Study
FLEX	Flexible Lifestyles Empowering Change Study
FPRM	Fat and Protein Rich Meal
G	Grams
GIP	Gastric Inhibitory Peptide
GLP-1	Glucagon-like Peptide 1
G/KG	Grams Per Kilogram Bodyweight
G/KG/Day	Grams Per Kilogram Bodyweight Per Day
GPAQ	Global Physical Activity Questionnaire
IRB	Institutional Review Board
IQR	Interquartile Range
KCAL	Kilocalories
KG	Kilograms
HBA1C	Hemoglobin A1c

HI1T	Metabolic, Hormonal, and Physiological Characterization of Isoenergetic High Intensity Interval Training and Moderate Intensity Continuous Training in Adults with Type 1 Diabetes Study
HIIT	High Intensity Interval Training
MDII	Multiple Daily Insulin Injections
MET	Metabolic Equivalents
MET-Min	Metabolic Equivalents x Minutes of Physical Activity
MICT	Moderate Intensity Continuous Training
MVPA	Moderate-to-Vigorous Physical Activity
N	Number of Participants
NORC	Nutrition Obesity Research Center
OBS	Number of Observations
OW/OB	Overweight or Obesity
PDPAR	Previous Day Physical Activity Records
PYY	Pancreatic Polypeptide Hormone
RDA	Recommended Daily Allowance
RPE	Rate of Perceived Exertion
SD	Standard Deviation
SM	Standard Meal
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
TAR	Percent Time Spent Above Range (>180mg/dL)
TBR	Percent Time Spent Below Range (<70mg/dL)
TIR	Percent Time Spent In Recommended Glucose Range (70-180mg/d)
UNC	University of North Carolina at Chapel Hill
VO_2	Volume of Oxygen Consumption
WHO	World Health Organization

CHAPTER 1. INTRODUCTION AND RESEARCH AIMS

1.1. Introduction

Exercise is central to diabetes management and the prevention of cardiovascular disease, which is the leading cause of morbidity and mortality among people with type 1 diabetes (T1D).[2,3] Despite the numerous benefits associated with regular exercise, as many as 60% of people with T1D report not participating in any structured exercise.[4] The fear of hypoglycemia, defined as a blood glucose below 70mg/dL, with exercise is the strongest barrier to regular physical activity in adolescents and adults with T1D.[5-11] Hypoglycemia can pose a serious medical emergency if not detected and treated promptly.[12,13] Following exercise, the risk of hypoglycemia remains elevated for at least 24 hours among people with T1D.[14-16] **There is a critical need for strategies that can reduce the risk of hypoglycemia with exercise and empower people with T1D to engage in greater levels of physical activity to improve their health and diabetes management.**

Recent expert consensus statements have provided guidance for carbohydrate intake before and after exercise to help manage glycemia during and following exercise, however, less is known about the role of protein intake on exercise-related glycemia.[17-20] Feeding studies in adolescents and adults with T1D have shown a significant and sustained increase in glycemia beginning 2-3 hours following protein intake which is sustained for at least 5 hours, with two studies demonstrating a protective effect against hypoglycemia among adolescents with T1D.[21-26] Most of these studies, however, observed the effect of protein on glycemia outside of the context

of exercise and only observed the effects of protein intake up to 5 hours after a meal, limiting our understanding of the effect past this time period. **Understanding the effects of protein consumed before or after exercise (peri-exercise) on exercise-related glycemia is essential to identification of nutritional strategies that may attenuate the risk of adverse glycemic excursions, improving the safety of exercise for people with T1D.**

To address this critical need, we proposed to assess the effect of pre- and post-exercise protein intake on glycemic control as measured by continuous glucose monitoring (CGM) time in glucose range (70-180mg/dL, TIR),[27] time below range (<70mg/dL, TBR) and time above range (>180mg/dL, TAR) over following exercise using data from 258 adolescents with T1D (Flexible Lifestyles Empowering Change (FLEX) Trial, NCT01286350, PI Mayer-Davis). Additionally, using data from the Metabolic, Hormonal, and Physiological Characterization of Isoenergetic High Intensity Interval Training and Moderate Intensity Continuous Training in Adults with Type 1 Diabetes (HI1T) study (NCT04664205, PI Smith-Ryan), we added to the rigor of our post-exercise protein intake analyses by assessing the effect of post-exercise protein intake on TIR, TBR, and TAR overnight following supervised exercise among 14 adults with T1D in the utilizing energy expenditure matched bouts of high intensity interval or moderate intensity continuous training following an overnight fast. The time-stamped diet, exercise, and CGM data provided by these studies uniquely positions us to evaluate a temporal relationship between pre- and post-exercise protein intake and glycemic control during and following exercise among adolescents and adults with T1D overnight during which they are at an increased risk of hypoglycemia. The following proposed aims will provide valuable information that will inform exercise nutrition practice for people living with T1D.

Aims 1 and 2: Estimate the effect of pre- and post-exercise protein intake on glycemic control during and following exercise (FLEX). Using matched, time stamped previous day physical activity records, 24-hour dietary recalls, and CGM data, we estimated the effect of protein intake within 4 hours prior to the onset of a bout of moderate-to-vigorous physical activity (MVPA) on TIR, TBR and TAR during **(Aim 1a)** and overnight following **(Aim 1b)** the cessation of a bout of MVPA defined as ≥30 minutes at a metabolic equivalent of ≥3 among adolescents with T1D. We also estimated the effect of post-exercise protein intake, consumed from the cessation of a bout of MVPA until midnight, on TIR, TBR, and TAR overnight following the cessation of each bout of MVPA among adolescents with T1D **(Aim 2)**. From the available data, approximately 454 observations from 114 participants meet our criteria. Mixed linear models were utilized to account for non-independence of observations due to repeated measures. We hypothesized that greater protein intake pre- and post-exercise will be associated with greater TIR and reduced TBR among adolescents with T1D.

Aim 3: Estimate the effect of post-exercise protein intake on glycemic control up to 24 hours following energy expenditure matched bouts of supervised exercise among adults with T1D (HI1T). 14 adults with T1D participated in supervised sessions of high intensity interval training and moderate intensity continuous training matched for energy expenditure separated by at least 7 days. CGM and accelerometry data were collected throughout the study and food records were collected for the day prior, day of, and day after each visit. We hypothesized that greater post-exercise protein intake will be associated with greater TIR and reduced TBR among adults with T1D. We sought to identify a unique intersection of nutrition and exercise that will inform exercise nutrition practice and open new pathways to develop

interventions that may help reduce the fear of hypoglycemia and empower individuals with T1D to safely achieve greater levels of physical activity.

CHAPTER 2. BACKGROUND AND MOTIVATION

2.1. Background on Type 1 Diabetes

2.1.1. Pathophysiology and Etiology

Type 1 diabetes (T1D), which accounts for approximately 5-10% of diabetes diagnoses, is characterized by the cellular-mediated autoimmune destruction of pancreatic β-cells, leading to the inability produce endogenous insulin.[28] The resulting insulin deficiency prevents tissues from taking up glucose from circulation leading to chronic hyperglycema and eventually ketoacidosis if not managed with exogenous insulin injections.[29] While the clinical presentation of T1D is often rapid, the rate of development of T1D can be variable as youth progress through three recognized stages of disease development. The first stage, which may last for months or years, is characterized by the presence of autoimmunity with asymptomatic normoglycemia followed by a second stage in which dysglycemia is present, but asymptomatic.[30] The final stage of T1D development is defined by the sudden onset of symptoms, such as polyuria, polydipsia, nocturia, enuresis, weight loos, or diabetes ketoacidosis, which commonly don't present until at least 90% of pancreatic β-cells have been destroyed.[29,30]

While the exact processes that underlie T1D risk remain unclear, there are multiple factors known to be etiologically related to T1D susceptibility including genetic, environmental and immunological factors.[29] Immunologically, autoantibodies GAD, IA2, IAA, and ZnT8 are serological markers of β-cell autoimmunity with IAA and ZnT8 most commonly expressed in

children younger than 10 and GAD and IA-2 more commonly expressed in older individuals and among females.[31,32] These autoantibodies are believed to be related to HLA-DR-DQ genotype which is contributes 30-50% of genetic risk for T1D.[33-36] The human leukocyte antigen (HLA) genotype are genes in major histocompatibility complexes (MHC) which code for proteins that help differentiate between one's own cells and foreign cells.[33-35] The DR and DQ alleles of the HLA genotype determine an individual's HLA-attributable genetic risk of developing T1D with individuals who are positive for HLA-DRB1*03 or DRB1*04 with DQB1*03:02 shown to have the highest risk of developing T1D.[33,35]The remaining genetic risk is attributed to over 60 other non-HLA genes with smaller effects, with genome-wide association studies identifying INS, PTPN22, CTLA4 and IL2RA genes contributing the highest risk of these non-HLA genes due to their role in the immune regulation of the pancreatic β-cell.[37,38]

While it is believed that environmental triggers may initiate pancreatic β-cell auto-immunity, the exact processes remain relatively unclear.[29] Enterovirus infection is believe to be a potential environmental trigger, especially if it occurs early in childhood, as it has been associated with pancreatic β-cell islet autoimmunity and T1D risk and enteroviruses have been detected in the islets of people with T1D.[39-44] Congenital rubella syndrome is believed to be another potential environmental pathogen which may be associated with the development of T1D with exposure to rubella during pregnancy resulting in diabetes in about 20% of cases.[45,46] Additionally, the TEDDY study has found that, among a cohort of babies at a high genetic risk of developing T1D, early infant feeding practices, such as the early introduction of gluten-containing cereals, probiotics and eggs may reduce the risk of developing islet autoimmunity.[47]

2.1.2. Epidemiology of Type 1 Diabetes

Type 1 diabetes is one of the leading causes of chronic disease in youth, with a prevalence estimated to include over 9 million people globally.[48,49] Among youth 19 years or younger in the United States in 2017, the prevalence of T1D was 2.15 per 1000 youth, representing a relative increase of 45.1% since 2001.[50] Additionally, the incidence of T1D in the United States has been increasing by approximately 1.9% per year from 2002-2015, with the greatest increases in incidence observed among black (2.7% per year), Hispanic (4.0% per year) and Asian and Pacific Islander (4.4% per year) youth compared to Non-Hispanic White youth (0.7% per year).[51] Globally, the incidence of T1D is highest in Northern European countries, including Finland, which also have a high prevalence of high risk alleles.[52,53] Type 1 diabetes is associated with numerous health complications including kidney disease, retinopathy, and peripheral neuropathy, in addition to a substantially elevated risk of cardiovascular disease.[54-59]

2.1.3. Intensive Insulin Therapy, Obesity, and Cardiovascular Disease Risk

Among people living with type 1 diabetes, the risk of developing cardiovascular disease is approximately ten times that of those without diabetes, making it the leading cause of morbidity and mortality among this population.[2] The landmark Diabetes Control and Complications Trial (DCCT/EDIC) demonstrated the extensive benefits of intensive insulin therapy which reduced the risk of cardiovascular disease events and mortality by 57% and 42%, respectively.[60] Intensive insulin therapy, however, was also been associated with excess weight gain compared to previously conventional therapies in the DCCT/EDIC trial. [61] Recent longitudinal studies have also shown that the prevalence of overweight and obesity among adolescents and adults with T1D has been increasing at a rate greater than in the general population which has been associated with intensive insulin therapy.[62,63] This is a concern for

those with type 1 diabetes as long-term follow up of participants in the DCCT/EDIC trial found that the benefits of intensive insulin therapy were substantially diminished among those classified as excessive gainers.[64] In fact, at 14 years follow-up there was no difference in the incidence of cardiovascular disease between those on intensive insulin therapy in the highest quartile of weight gain and those who did not receive intensive insulin therapy highlighting the importance of weight management in conjunction with intensive insulin therapy among people with T1D.[65]

2.2. Background on Physical Activity Among People with Type 1 Diabetes

2.2.1. Physical Activity Recommendations for People Living with Type 1 Diabetes

The American Diabetes Association currently recommends at least 150 minutes of moderate-to-vigorous physical activity (MVPA) per week among adults with T1D and at least 60 minutes per day among children and adolescents, which is similar to our national guidelines and the World Health Organizaton guidelines for the general population.[66-68] Despite the benefits associated with regular exercise, adherence to recommended physical activity levels is low among people with T1D. In a large, multicenter study of 18,028 adults with T1D, over 60% of participants reported not participating any regular recreational exercise.[4] In another study among adults with T1D which utilized accelerometry to quantify weekly levels of physical activity, only 32% of adults with T1D obtained recommended levels of physical activity.[11] Similarly, children and adolescents with T1D engage in less physical activity than their peers without diabetes, with only 37.8% achieving recommended levels of physical activity.[69]

2.2.2. Role of Physical Activity on Cardiovascular Health, Obesity, and Glycemia Among People With Type 1 Diabetes

Physical activity plays a central role in the promotion of cardiovascular health and weight management for people with T1D. In a large multicenter study of 18,028 adults with T1D, greater frequency of physical activity was inversely associated with HbA1c and body mass index (BMI) as well as diabetes complications and cardiovascular risk factors including dyslipidemia, hypertension, retinopathy, microalbuminuria, and diabetic ketoacidosis.[4] Additionally, in a recent study among adults with T1D which incorporated more objective measures of physical activity (accelerometry), greater weekly physical activity levels were associated with reduced HbA1c, BMI, waist circumference and fat mass, but also with an elevated number of hypoglycemic events.[11] In both of these studies, however, fewer than 40% of adults with T1D achieved recommended levels of physical activity.[4,11] Similarly, among youth and adolescents with T1D, several observational and multi-center studies have shown that free-living levels of MVPA are associated with improved blood lipid profiles and insulin sensitivity, reductions in adiposity and HbA1c, as well as reduced insulin dosing requirements.[70-75]

Intervention studies in youth and adults with T1D have confirmed many of the benefits observed in observational studies. In two meta-analyses which reviewed up to 26 studies which assessed the role of physical activity on at least one health outcome among individuals with T1D younger than 18 years old, significant benefits of physical activity were found for HbA1c, BMI, triglycerides, and total cholesterol, however, they did not report on the frequency of hypoglycemia in these studies.[76,77] While fewer intervention studies have been published among adults with T1D, a meta-analysis which included six intervention studies among adults with T1D found significant decreases in HbA1c and significant improvements in cardiorespiratory fitness

associated with exercise interventions.[78] Additionally, in one study included in the meta-analysis, the exercise intervention significantly reduced in insulin dose among adults with T1D with elevated HbA1c.[79]

2.2.3. Barriers to Physical Activity Among People with T1D

People with T1D experience many of the same barriers to physical activity as their healthy peers including lack of time, motivation, or access to facilities and low fitness levels, however, they also face barriers specific to diabetes.[7-9] Barriers specific to people with T1D include diabetes-related stigma, knowledge and confidence about managing the effects of exercise on glycemia, and the fear of exercise-related hypoglycemia.[5-11] Dysglycemia with exercise, specifically the fear of hypoglycemia, is a prevalent barrier to physical activity that has been shown to persist across age groups of people with T1D.[5-11]

Studies in both adolescents and adults with T1D have found that the fear of hypoglycemia is the strongest barrier to exercise.[5-11] Depletion of muscle and liver glycogen during exercise contributes to increased insulin sensitivity, which elevates the risk of hypoglycemia for at least 24 hours following exercise.[16,19] Of particular concern is an increased risk of hypoglycemia overnight, which often results in more severe episodes of hypoglycemia or diabetic ketoacidosis.[80-82] The glycemic response to exercise can vary based on a number of influences including the blood glucose concentration before exercise, the amount of insulin in circulation, the composition of the previous meal, or the intensity and duration of exercise, making adverse glycemic excursions difficult to predict.[83]

2.2.4. Proposed Mechanisms Underlying Adverse Glycemic Excursions With Exercise

During aerobic exercise in individuals with and without diabetes, glucose uptake into skeletal muscle can increase by as much as 50-fold independent of the effects of insulin.[84] In people without diabetes, however, insulin secretion decreases and the secretion of counter-regulatory hormones, such as glucagon and epinephrine, increases with aerobic exercise, allowing for glucose to be maintained in a relatively stable range.[85] In people with T1D, however, exogenously provided insulin levels cannot be rapidly decreased as endogenously produced insulin can in those without T1D and may actually increase due to greater blood flow to injection sites may mobilize more insulin into circulation.[86,87] Elevated insulin concentration relative to counter-regulatory hormones increases glucose disposal and may decrease lipolysis, further increasing the reliance on glucose during exercise.[85,88] As such, blood glucose tends to drop during aerobic exercise among people with T1D, often leading to hypoglycemia within about 45 minutes of the start of exercise unless additional carbohydrate is provided.[14,15,89] While glucose uptake begins to decrease following exercise, increased sensitivityto insulin, which is experienced by both individuals with and without T1D, can cause glucose disposal to remain elevated for at least 24 hours following exercise, increasing the risk of hypoglycemia among individuals with T1D during that recovery period.[16,90]

It's also important to note that the glycemic response to exercise may differ by the intensity and duration of the exercise performed. Due to the intensity of anaerobic types of exercise, such as resistance training or high intensity interval training, skeletal muscle relies more heavily on muscle phosphate, glycogen and lactate, as these metabolic substrates can be utilized more quickly to fuel the high energy demands of the activities.[91] While glucose is relied on more heavily for these types of activities, this type of exercise also tends to be shorter in

duration due to the intensity of the exercise. Anaerobic exercise has also been shown to cause a larger counter-regulatory response leading to greater endogenous glucose production, typically causing blood glucose levels to rise during these types of exercise.[83,92-95] While the effects of anaerobic exercise on late-hypoglycemia are mixed, the majority of studies have indicated that the risk of late-onset hypoglycemia does not differ between aerobic and anaerobic exercise[96-99], however, awareness of hypoglycemia may decrease following high intensity activity, potentially due to differences in lactate production with high-intensity exercise which may serve as an alternative fuel for the brain during hypoglycemia.[100,101]

2.2.5. Current Approaches to Management of Glycemia with Exercise

Current approaches to managing glycemia with exercise focus on managing starting glycemia or adjusting peri-exercise carbohydrate intake or insulin dosing. A recent consensus report from leading experts in exercise physiology and diabetes management provided guidelines for glycemic targets for exercise for people with T1D.[19] These guidelines provide differing recommendations based on the modality of exercise. As anaerobic exercise tends to increase blood glucose during exercise, blood glucose levels below 270 mg/dL (15mmol/L) are generally considered safe to begin anaerobic types of exercise. However, as blood glucose levels may rise, an increased basal rate may help to prevent or treat hyperglycemia during or immediately following exercise among individuals using continuous subcutaneous insulin infusion (CSII) when starting glycemia is greater than 180 mg/dL (10 mmol/L) .[19] Alternately, it is recommended that individuals with T1D planning to participate in aerobic exercise aim for a starting glycemia of 126-180 mg/dL (7-10 mmol/L), with a recommendation of consuming 10-20g of carbohydrate if glycemia is below this level.[19]

Furthermore, when aerobic exercise occurs within 120 mins of a meal, a 25-75% reduction, depending on the intensity of exercise, in bolus insulin is suggested to reduce the risk of hypoglycemia during exercise.[19,102-104] Among those using CSII, a reduction of up to 100% of basal rate starting up to 90 minutes prior to exercise is recommended with resumption of normal rates at the end of exercise.[19,105-108] Additionally, a 20% reduction in basal rate overnight is recommended following afternoon exercise to prevent nocturnal hypoglycemia.[19,104,109] To optimize glycemia during and following endurance exercise, it is also recommended for people with T1D to consume a meal containing at least 1 gram of carbohydrate per kilogram of body weight at their pre-exercise meal and at least 1.0 – 2.0 g of carbohydrate per kilogram at the meal following exercise to replenish muscle glycogen, prevent hypoglycemia and maximize recovery from exercise.[110,111] For prolonged endurance exercise, consumption of 30-60g/h of carbohydrate for durations lasting 60-150 minutes or 60-90g/h of carbohydrate for durations >150 minutes is recommended for improved performance and to prevent hypoglycemia.[110,111]

2.2.6. Current Gaps in Existing Strategies for Management of Exercise-Related Glycemia

The guidelines set out by the expert consensus statement provide a first step in supporting people with T1D in participating in greater levels of physical activity; however, the role of protein intake in supporting exercise-related glycemia is not considered in these guidelines. This is an important gap in our current guidelines as people who choose to exercise for the purpose of weight management have reported that the need to consume carbohydrate to prevent hypoglycemia can create a feeling of futility around exercise.[6] Additionally, the physical benefits of exercise have been demonstrated to be an important facilitator which encourages greater levels of physical activity among people with T1D.[7,112] Nutritional strategies which promote both a reduced risk of exercise-related hypoglycemia and enhancements in the exercise-related

improvement in fitness and body composition, may provide more comprehensive support to people with T1D, empowering them to improve their health and well-being through more regular physical activity.

2.3. Sports Nutrition Guidelines on Protein Intake and Timing to Improve the Adaptive Response to Exercise

Protein intake is a common nutrient of focus for sports nutrition strategies due to its central role in recovery, muscular hypertrophy and weight management. While the recommended daily allowance for protein intake is 0.8 grams per kilogram per day, current sports nutrition guidelines recommend protein intakes ranging from 1.2 – 2.0 grams per kilogram of body weight per day to facilitate recovery and enhance the adaptive response to exercise.[113-116] Increasing protein intake post-exercise has a synergistic effect with exercise, enhancing muscle protein synthesis and decreasing muscle protein breakdown, leading to a net positive protein balance.[117-119] When paired with resistance exercise, increased protein intake has been demonstrated to improve muscular strength and hypertrophy and reduce recovery time[120,121] and when paired with endurance exercise it has been demonstrated to improve recovery and reduce muscular soreness.[121-123] Furthermore, protein intakes as high as 1.8-2.7g/kg/day are recommended for individuals in an energy deficit[116,121,124] as, when paired with a moderate hypocaloric diet (-500kcal) and exercise, increasing protein intake has been shown to promote greater reductions in fat mass and greater retention of lean mass.[125-127]

The timing of protein intake around exercise is also an important consideration for promoting optimal adaptation to exercise. Current sports nutrition guidelines recommend a pre-exercise meal high in carbohydrate within 4 hours prior to exercise.[116,121,128] While guidelines focus on carbohydrate, they also suggest that the addition of protein to a pre-exercise meal may

enhance protein synthesis during exercise and reduce muscle protein breakdown which may support post-exercise recovery.[123,129-131] Sports nutrition guidelines also recommend protein doses of approximately 0.25-0.3g/kg of body weight immediately following exercise as well as throughout the day following exercise, ideally every 3-4 hours, as effective strategies to enhance the adaptive response to exercise by taking advantage of the increased sensitization of muscle to the protein synthetic response of dietary protein which is highest during and immediately following exercise, but remains elevated for 24-72 hours after exercise.[18,116,121,128,129]

While this dissertation work focuses on the potential glycemic benefits of peri-exercise protein intake for people with T1D, it is important to note the potential dual benefit that could be experienced by people with T1D if peri-exercise protein intake proves to be an effective strategy in mitigating adverse glycemic excursions during and following exercise.

2.4. Background on The Effects of Dietary Protein Intake on Glycemia in Type 1 Diabetes

While carbohydrates are the predominate macronutrient affecting post-prandial blood glucose, protein and fat have also been shown to contribute to post-prandial increases in glycemia outside of the context of exercise. In children with T1D, meals high in protein or fat were each separately found to increase post-prandial blood glucose from 3 hours to 5 hours post-meal, though only the high protein meal was found to be protective against hypoglycemia during the 5 hours following the meal among those children.[24] Other studies among children, adolescents, and adults with T1D have shown similar trends in post-prandial glycemia following high protein intakes with glucose peaking around 3.5 hours following a meal and remaining elevated past 5 hours.[21,22,25,132,133] Paterson et al[23] further demonstrated a dose-response relationship of protein intake with increasing glycemia 3-5 hours post-prandial for mixed meals containing at least 12.5 grams of protein.

While most studies assessing the effects of protein on glycemia in T1D have limited glycemic observations to 5 hours following a meal, a study in adolescents with T1D observed blood glucose levels overnight for 12 hours after a fat and protein rich meal (FPRM) compared to a standard meal (SM) with identical carbohydrate content and insulin dose and found significantly greater glucose area under the curve 4 to 12 hours after a FPRM[134]. In this study, while carbohydrate was consistent between the FPRM and standard meal, the protein and fat in the FPRM were double that in the standard meal making it difficult to distinguish whether the relative contribution of the protein or the fat content on the observed glycemic response, however, it does suggest that the effects of protein on glycemia may persist past the 5 hours reported in most studies.[134]

2.5. Peri-Exercise Protein Intake as an Innovative Approach to Reduce the Risk of Adverse Glycemic Excursions

Literature on nutritional strategies for managing risk of dysglycemia during and following exercise is scarce and has focused predominantly on carbohydrate intake.[83,104,135-139] In these studies, consuming carbohydrate prior to or during exercise has been shown to reduce the risk of hypoglycemia during and for up to 6 hours following exercise, with greater benefits observed when carbohydrate intake is paired with bolus or basal insulin adjustments.[83,135-140] The optimal amount of carbohydrate prior to exercise, however, depends upon the amount of insulin on board and the starting blood glucose levels prior to exercise as well as the duration and intensity of exercise being performed with only small amounts of carbohydrate (10-20g) necessary for shorter duration (30 minutes) bouts of moderate intensity exercise, but higher amounts (75-90g/hour of exercise) necessary for longer duration or more vigorous exercise.[135,137-139] Additionally, current guidelines recommend a meal with at least 1.0g/kg of

bodyweight following exercise to help replenish glycogen levels and reduce the risk of late-onset hypoglycemia following exercise.[19] Very few studies, however, have examined the role of protein intake on exercise-related glycemai.[26,141]

Dube et al. compared the effects of a standardized breakfast + placebo drink, standardized breakfast + pre-exercise carbohydrate beverage, and a protein-supplemented breakfast on glycemia during exercise two hours after breakfast in adolescents with T1D.[26] Their results showed that the protein-supplemented meal prior to exercise was equally effective in reducing the frequency of hypoglycemia during and immediately following exercise as the pre-exercise carbohydrate beverage.[26] While the results of this study supported the potential efficacy of pre-exercise protein intake as a strategy for managing dysglycemia during and immediately following exercise in adolescents with T1D, the study had a small sample size (n=10) and only assessed glucose levels up to 60 minutes following exercise, which is a limitation as we know the risk of hypoglycemia remains elevated for at least 24 hours following exercise in people with T1D.

Additionally, in a brief report by Paramalingam et al., the effects of post-exercise protein intake on blood glucose requirements required to maintain euglycemia overnight were examined.[141] In this study, participants consumed either 50g of whey protein or a water placebo following 45 minutes of moderate-intensity cycling exercise performed in the afternoon. A euglycemic clamp was used to maintain euglycemia overnight and glucose infusion rates, glucagon, GLP-1 and GIP levels were assessed during this time. Glucose requirements to maintain euglycemia overnight were significantly lower and Glucagon, GLP-1 and GIP levels were significantly higher following post-exercise protein compared to water suggesting that post-exercise protein intake may reduce the risk of hypoglycemia via glucagon-mediate pathways.[141]

This study, however, was limited by a small sample size (n=6) and it's unclear if these effects may differ when protein is consumed as part of a mixed meal post-exercise.

2.6. Motivation for Dissertation Studies

2.6.1. Identified Clinical Needs and Gaps in Evidence

Physical activity plays a central role in both weight management and cardiovascular disease risk, which is the leading cause of morbidity and mortality among people with T1D.[2] Despite the benefits of physical activity, the majority of youth and adults with T1D don't achieve recommended levels of physical activity.[4,11,69] The strongest barrier to participating in physical activity among both youth and adults with T1D is the fear of exercise-related hypoglycemia.[5-11] While nutritional recommendations on peri-exercise carbohydrate intake are published, less is known about the role of other nutrients in exercise-related glycemia.[19] Additionally, the need to consume carbohydrate to prevent hypoglycemia with exercise may create a feeling of futility among people with T1D, especially when managing weight is the motivator for participating in exercise.[6] Nutritional strategies are needed which can support both the safety and adaptive benefits of exercise to empower people with T1D in engaging in more regular physical activity.

2.6.2. Rational and Potential Public Health Impact of Peri-Exercise Protein Intake For Addressing Exercise-Related Dysglycemia Among People With Type 1 Diabetes

Following sports nutrition guidelines for dietary protein intake may be a promising nutrition approach to mitigating the risk of hypoglycemia during and following exercise while also potentially enhancing the benefits of exercise. Feeding studies in people with T1D have shown that meals high in protein cause a mild, but prolonged hyperglycemic effect which persists for at least 5 hours and possibly longer with higher doses. In theory, following sports nutrition guidelines for dietary protein intake may help to mitigate the risk of hypoglycemia

during and following exercise by causing a mild, but prolonged increase in glycemia which may counteract declining glycemia associated with exercise. While research examining the role of protein intake on glycemia in the context of exercise are severely limited, two studies have examined the effects of pre- or post-exercise protein intake on glycemia and found preliminary evidence of its potential benefits on reducing the risk of hypoglycemia during and following exercise.[26,141]

This research has important public health implications as peri-exercise protein intake poses a potential nutritional strategy which may help to not only address the leading barrier to physical activity among people with T1D, but also potentially enhance the benefits of exercise providing increased motivation to engage in greater levels of physical activity. The availability of time-stamped dietary intake, continuous glucose monitoring and physical activity data in two different populations with T1D provides a unique opportunity to assess the consistency of the relationship of peri-exercise protein intake on exercise-related glycemia in two different populations of people with T1D and reveal important factors which may contribute to diverging responses. Additionally, the results of this study will help to inform the development of updated exercise nutrition guidelines for people with T1D, as current guidelines are limited in their ability to provide nutrition guidance to support exercise outside of carbohydrate and insulin dosing. Finally, the results of this dissertation research will inform the development of future interventional studies which can more robustly investigate the role of protein intake on exercise-related glycemia.

2.6.3. Key Research Questions

There are three key research questions which are addressed in the following chapters.. Chapter 3 utilizes data from the FLEX study to address the research question: Is elevated protein intake within 4 hours prior to a bout of MVPA associated with improved glycemia during and following MVPA among adolescents with T1D? Chapter 4 utilizes the same dataset to address the research question: Is elevated post-exercise or daily protein intake associated with improved glycemia following bouts of MVPA among adolescents with T1D? Finally, Chapter 5 uses data from the HI1T study to ask the research question: Is elevated post-exercise protein intake associated with improved glycemia following exercise among adults with T1D?

CHAPTER 3. PRE-EXERCISE PROTEIN INTAKE IS ASSOCIATED WITH REDUCED TIME IN HYPOGLYCEMIA AMONG ADOLESCENTS WITH TYPE 1 DIABETES

3.1. INTRODUCTION

For adolescents living with type 1 diabetes (T1D), regular physical activity improves hemoglobin A1c, cardiorespiratory fitness, body mass index, insulin sensitivity, lipid profiles, and perceived well-being. [76,78,142] The risk of hypoglycemia with physical activity, however, is a major barrier to regular participation in physical activity among this population. [8,143] Nutritional strategies that may reduce the risk of hypoglycaemia during physical activity would be invaluable in promoting greater levels of physical activity among adolescents with T1D which may then lead to improvements in health and well-being.

Nutrition guidelines for carbohydrate consumption prior to physical activity have been established [19]; however, less is known about the role of pre-exercise protein intake on glycemia during or following physical activity. Sports nutrition guidelines suggest that adding protein to a carbohydrate containing meal within 4 hours prior to exercise may promote improved recovery from a proceeding exercise bout by promoting increased muscle protein synthesis and reduced muscle protein breakdown during exercise. [116,121,128,129] Among adolescents and children with T1D, protein ingestion has also been shown to induce a mild, prolonged increase in glycemia lasting up to five hours following a meal, with one study showing higher glucose concentrations as much as 12 hours following fat and protein rich meals among adolescents with T1D. [23,24,133,134] In theory, this hyperglycemic effect of protein, if consumed prior to exercise as suggested by

sports nutrition guidelines, may help to mitigate declining glycemia during and following physical activity among adolescents with T1D.

To the authors' knowledge, only one study has examined the effect of pre-exercise protein intake on glycemia among adolescents with T1D [26]. In this study, the authors monitored blood glucose levels during 60 minutes of moderate-intensity cycling exercise following either a protein supplemented breakfast consumed two hours prior to exercise, a standard breakfast with a carbohydrate supplement 15 minutes prior to exercise, or a standardized breakfast with a placebo drink prior to exercise. They found that consumption of a protein-supplemented breakfast two hours prior to exercise was equally effective in reducing the risk of hypoglycaemia during exercise compared to a standardized breakfast which was supplemented with a carbohydrate beverage consumed 15 minutes prior to exercise (n=10).[26] To the authors' knowledge, however, no previous studies have investigated whether pre-exercise protein intake may improve glycemia in the hours following physical activity. This is an important gap in the literature as the risk of hypoglycemia is elevated for up to 24 hours following activity in people with T1D.[16] As the effects of protein on glycemia have been shown to persist for at least 5 and possibly as long as 12 hours, [24,134] it's possible that pre-exercise protein may have benefits that persist past the cessation of physical activity.

As such, this study aimed to investigate the relationship between protein intake consumed within 4 hours prior to a bout of moderate-to-vigorous physical activity (MVPA) on the percent of time spent in recommended glucose range (TIR, 70-180mg/dL), percent of time spent above range (TAR, >180mg/dL) and percent time spent below range (TBR, <70mg/dL) during (Aim 1) and until the following morning (Aim 2) among adolescents with T1D. We hypothesized that

pre-exercise protein intake will be associated with reduced TBR and improved TIR during and following bouts of MVPA.

3.2. METHODS

3.2.1. Study Design

Data was analysed from the Flexible Lifestyles Empowering Change (FLEX) study[144,145] (1UC4DK101132-01), a randomized controlled trial of a behavioral intervention aimed at improving glycemia, psychosocial and metabolic outcomes among adolescents with T1D. The FLEX study was reviewed and approved by institutional review boards at clinical sites in Colorado and Ohio as well as at the coordinating site, the University of North Carolina at Chapel Hill. From 05/01/2014 to 04/04/2016, 258 adolescents between the ages of 13-16 years were enrolled in the FLEX study and randomized to receive either an 18-month adaptive behavioural intervention (n=130) or a usual care control (n=128). Parents provided written informed consent and adolescents provided written assent. The adaptive behavioural intervention incorporated motivational interviewing and problem-solving skills training to promote adherence to T1D self-management skills, insulin dosing, blood glucose testing, diet, and physical activity behaviors. The participants' diabetes self-management strategies were determined through the motivational interviewing process, with an emphasis on glucose control. The intervention did not systematically incorporate guidance for increasing physical activity. These post-hoc analyses utilize secondary measures from baseline and 6-month visits from the FLEX study to assess the proposed aims. Full details of the design and main results of the FLEX study have been previously published. [144,145]

3.2.2. Participants

Participants for the FLEX study were enrolled from two sites: Barbara Davis Center for Childhood Diabetes in Colorado and Cincinnati Children's Hospital and Medical Center in Ohio, coordinated by the University of North Carolina (UNC) at Chapel Hill. Eligible participants were aged 13-16 years at study entry and had HbA1c 8-13% and duration of diabetes >1 year. Youth that were pregnant or had a concurrent severe physical (e.g. Cancer), developmental (e.g. cognitive impairment) or psychiatric (e.g. severe psychopathy) medical condition were excluded from participating in the FLEX study. The distribution of baseline demographic, clinical, glycemic, dietary, and physical activity characteristics among participants included in the final analyses were evaluated and are provided in **Table 1**. Continuous variables are reported as mean and standard deviation except for non-normally distributed variables, in which median and interquartile range were reported. Categorical variables are described with counts and percentages.

3.2.3. Measures

Demographics and Health History

Participants completed demographic and health history questionnaires at baseline, 6 months, and 18 months following their baseline visit. From these questionnaires, self-reported age, sex and race/ethnicity were reported as well as insulin regimen, total previous day insulin dose, and T1D duration (years).

Physical Activity

Two previous day physical activity records (PDPAR) were collected in conjunction with the 24-hour dietary recalls over the phone. The previously validated PDPAR [146,147] divides the day into half-hour time blocks and queries the dominant activity and the approximate intensity of that activity for that period, categorized as ‘‘very light (slow breathing, little or no movement)’’, “light (normal breathing, regular movement)”, ‘‘medium (increased breathing, moving quickly for short periods of time)’’ or ‘‘hard (hard breathing, moving quickly for 20 minutes or more)’’ Each activity and perception of effort was matched to a corresponding MET value utilizing the Compendium of Physical Activities [148], as detailed by Weston et al. [147] From these records, bouts of MVPA were defined as 30 minutes or greater of physical activity at a MET value of greater than or equal to 3 METs.

Continuous Glucose Monitoring

Participants were provided with a blinded Medtronic iPro2 continuous glucose monitor (CGM) with the Enlite sensor for a 7-day period at baseline, 6-, and 18-months following the baseline visit. To enhance compliance and improve quality of CGM data collection, an iPro2 compatible meter (OneTouch Ultra2) was provided to the participant along with test strips (50 strips) for the 7-day CGM study period for calibration for testing 1- and 3 hours after insertion, pre-meal and before bed. Percent of time in recommended glucose range (TIR, 70-180mg/dL), time above range (TAR, >180mg/dL), and time below range (TBR, <70mg/dL) were calculated for the time period during a bout of MVPA (Aim 1) as well as from the end of a bout of MVPA until 6:30 AM the following morning (Aim 2) utilizing consensus report definitions of TIR, TAR, & TBR. [27] As the minimal reportable duration of a bout of activity with the PDPAR [146,149] is 30 minutes and previous research on the effects of protein intake on glycemia among

adolescents with T1D has demonstrated hyperglycemic effects lasting at least 5 hours [23,24,133,134], these analyses were restricted to observations with at least 30 minutes of CGM data during bouts of MVPA and at least 5 hours of CGM data following those bouts. An example timeline of exposures and outcomes is provided in **Figure 3.1.**

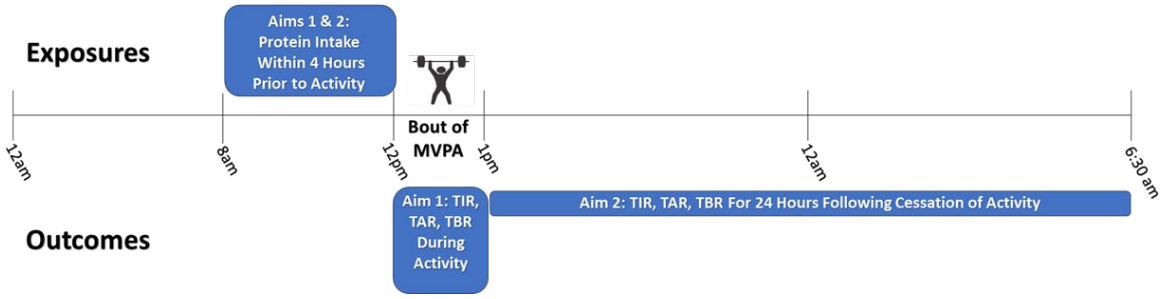

Figure 3.1. Timeline of Exposures and Outcomes Relative to a Bout of Moderate-to-Vigorous Physical Activity Using Multiple Measurements (Baseline & 6 Months)

Dietary Intake

Two unannounced 24-hour dietary recalls were collected at baseline and 6 months by phone during the 7-day CGM wear time by certified interviewers from the UNC NIH/NIDDK Nutrition Obesity Research Center staff (P30DK056350, MPI Mayer-Davis, Shaikh), using the Nutrient Data System for Research software and the multiple pass interviewing method.[62,150] Protein intake consumed within four hours prior to a bout of MVPA was quantified and represents the primary exposure for these post-hoc analyses. For these analyses, observations with pre-exercise protein intake greater than 3 standard deviations above the mean (>71.6g) were excluded as potential outliers. Furthermore, to account for the glycemic effect of carbohydrate and bolus insulin levels, pre-exercise carbohydrate intake (grams) was considered as a potential covariate in the Aim 1 analyses and daily carbohydrate (grams) intake was considered as a potential covariate in the Aim 2 analyses.

Anthropometrics & Body Composition

Height, weight, and natural waist were measured at baseline, 6- and 18-months after their baseline visit utilizing a wall-mounted stadiometer, calibrated electric scale, and a flexible fiberglass or steel tape measure, respectively. Height and weight measurements were also used to determine BMI. These measures were used to estimate percent bodyfat using validated age, race, and sex specific equations. [151] Estimated percent body fat was considered as a potential covariate in our statistical models.

3.2.4. Statistical Analysis

All statistical analyses were performed using SAS 9.4 (Cary, NC). Observations with incomplete dietary, physical activity, continuous glucose monitoring or covariate data were excluded in these post-hoc analyses as detailed in **Figure 3.2.** Potential sources of selection bias were explored by comparing exposure, covariate, and baseline glycemic data between those with and without adequate data using unadjusted mixed effects models to account for repeated measures. Mixed effects regression models assessed the relationship between protein intake within 4 hours prior to a bout of MVPA and TIR, TBR, TAR during a bout of MVPA (Aim 1) and until 6:30 AM the morning following a bout of MVPA (Aim 2). The estimated effect of protein intake (grams & grams/kg bodyweight) on glycemia was assessed utilizing a categorical variable to account for non-linearity. Categories for grams of protein intake were defined as <10 grams, 10-19.9 grams, and ≥20 grams of protein. Categories for g/kg were defined as <12.5g/kg, 0.125-0.25g/kg, and ≥0.25g/kg bodyweight. In both sets of analyses, non-consumers of protein during the 4 hours preceding the exercise bout were chosen as a reference group. These categories were based on sports nutrition recommendations which suggest a protein intake of 0.25g/kg or an absolute dose of 20-40g as an optimal level to promote positive adaptations to

exercise. [121,128] To assess whether smaller doses may be effective in promoting improved exercise-related glycemia, we chose to create additional categories above and below half of the dose recommended by sports nutrition guidelines.

Covariates were introduced into our models in groups: design (randomization assignment, study site), demographics (age, sex, race/ethnicity), clinical (duration of diabetes, insulin regimen, total previous day insulin dose, insulin dose per kilogram), body composition (estimated body fat percentage), physical activity (average bout METs, bout duration (mins), bout volume (MET-minutes), other daily physical activity (MET-minutes)), dietary (pre-exercise carbohydrate intake) and timing variables (hours until midnight). Covariates that produced a ≥10% change in the effect estimate or standard error were included in our final models.

3.3. RESULTS

3.3.1. Final Sample Size

Of a total of 645 MVPA bouts identified from 135 FLEX participants, 447 bouts from 112 participants were included in our final analytic models as detailed in **Figure 3.2.** In sensitivity analyses, there were no significant differences between those with sufficient CGM data and those with missing or insufficient CGM data in pre-exercise or daily nutrient intake, weight, BMI-z score, baseline HbA1c, or any other covariate included in our statistical models.

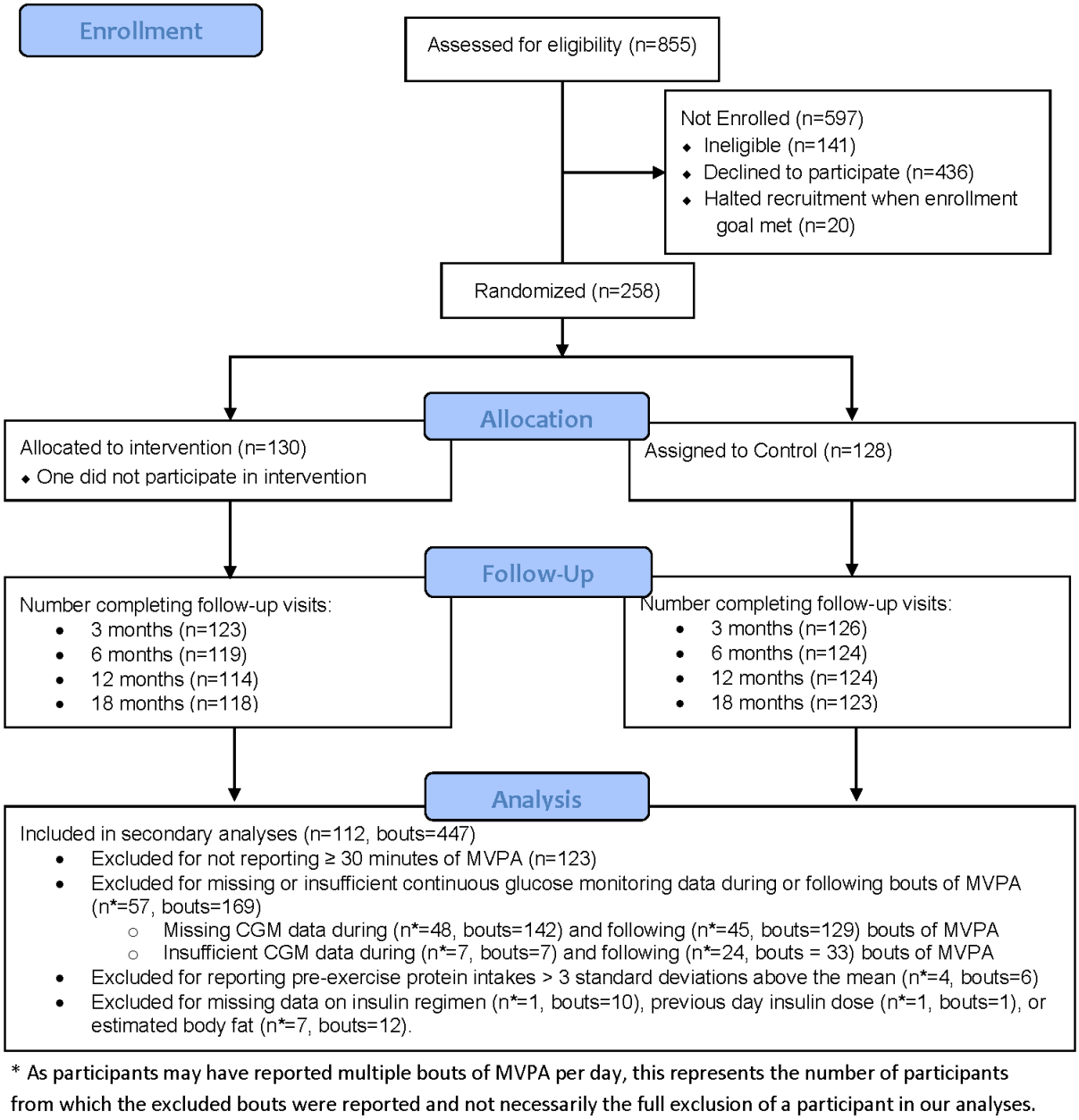

Figure 3.2. Consort Flow Diagram for Secondary Analyses of the Flexible Lifestyles Empowering Change (FLEX) Randomized Trial

3.3.2. Baseline Characteristics

Baseline characteristics of participants included in our analyses are provided in **Table 3.1**. The median age of participants included in these analyses was 14.5 (IQR: 13.8, 15.7), the median diabetes duration was 5.5 (IQR: 3.1, 9.0) years, and there was a relatively equal inclusion of male and female participants (53.6% female). Furthermore, while 73.0% of participants reported using an insulin pump for their diabetes care, 69.6% reported not having used a personal CGM for their diabetes care in the past 30 days. The participants spent on average 36.5% ± 13.7% TIR, 59.5% ± 16.1% TAR, and 2.1% (IQR: 0.4%, 5.6%) TBR per week at baseline.

Table 3.1: Baseline Characteristics of FLEX Participants Included in Final Analyses (n=112)

Demographic	Mean ± SD or N (%)
Age	14.5 (13.8, 15.7)
Female	60 (53.6%)
Male	52 (46.4%)
Race/Ethnicity	
Non-Hispanic White	90 (80.4%)
Non-Hispanic Black	2 (1.8%)
Hispanic	14 (12.5%)
Multiracial/Other	6 (5.4%)
Maximum Education of Parents	n=111
High School or Less	11 (9.9%)
Some College	30 (27.3%)
Four Year College Degree	49 (44.1%)
Graduate Degree	21 (18.9%)
Clinical	
Diabetes Duration	5.5 (3.1, 9.0)
Insulin Pump User (n=111)	81 (73.0%)
Previous Day Insulin Dose (units/kg) (n=110)	1.0 ± 0.3
Anthropometric	
Weight (kg)	58.8 (51.7, 70.0)
BMI Z-Score	0.7 ± 0.9
Estimated Body Fat %	28.1 (20.0, 33.1)
Glycemia	
No Personal CGM Use in Past 30 Days (n=102)	71 (69.6%)
Baseline HbA1c (%)	9.3 (8.5, 9.9)
Percent Time in Range (n=106)	36.5 ± 13.7
Percent Time Below Range (n=106)	2.1 (0.4, 5.6)
Percent Time Above Range (n=106)	59.5 ± 16.1
Diet	
Daily Caloric Intake (kcal)	1721.0 ± 560.1
Percent of Daily Calories from Protein	16.0 ± 3.5
Percent of Daily Calories from Carbohydrate	49.0 ± 7.7
Percent of Daily Calories from Fat	36.2 ± 6.4
Daily Fiber Intake (grams)	13.1 (10.1, 18.1)
Physical Activity (n = 108)	
Meet ADA Guidelines of ≥60mins MVPA/day	99 (91.7%)
Daily Minutes of MVPA	157.5 (105.0, 225.0)
Daily Minutes of Vigorous Physical Activity	45.0 (0.0, 90.0)

Continuous variables are reported as mean and standard deviation except for non-normally distributed variables, in which median and interquartile range are reported. Categorical variables are described with counts and percentages.

3.3.3. Effects of Absolute & Relative Protein Intake Within 4 Hours Prior to a Bout of MVPA on Glycemia During Physical Activity

The median protein intake within 4 hours prior to a bout of MVPA was 14.0 (IQR: 5.0, 26.3) grams or 0.23 (IQR: 0.08, 0.41) grams/kg of bodyweight. The mean TIR, TAR, and TBR during MVPA bouts was 34.50% ± 41.64%, 62.37% ± 43.53%, & 3.13% ± 13.99%, respectively. We observed that protein intakes of 10-19.9g and ≥20g compared to no protein intake were associated with a -4.41% (95% CI: -8.57%, -0.25%) TBR and -4.83% (95% CI: -9.00%, -0.66%) TBR, respectively (**Table 2**). Similarly, protein intakes of 0.125 – 0.249g/kg and ≥0.25g/kg compared to no protein intake were associated with -5.38% (95% CI: -9.63%, -1.13%) TBR and -4.32% (-8.27%, -0.38%) TBR, respectively (**Table 3**). No associations were observed between any category of absolute or relative protein intake and TIR or TAR during bouts of MVPA.

3.3.4. Effects of Absolute & Relative Protein Intake Within 4 Hours Prior to a Bout of MVPA on Glycemia From Cessation of Physical Activity Until 6:30 AM the Following Morning

The mean duration of time from the end of MVPA bouts until 6:30 AM the following morning was 15.56 ± 3.89 hours and the mean TIR, TAR, and TBR during this time was 40.80% ± 24.84%, 55.58% ± 28.00%, & 3.6% ± 8.33%, respectively. No association was observed between absolute (**Table 4**) or relative (**Table 5**) categories of protein intake and TIR, TAR, or TBR from the cessation of physical activity until 6:30 AM the following morning. Estimated associations ranged from -0.24% - 1.72% ($p>0.52$), -1.16% - 0.30% ($p>0.33$), -1.47% - 0.50% ($p>0.68$) for TIR, TBR and TAR, respectively across absolute and relative intakes of protein pre-exercise.

Table 3.2. Results of Mixed Effects Regression Models Estimating the Association Between Protein Intake (grams) Consumed Within 4 Hours Prior to a Bout of Moderate-to-Vigorous Physical Activity and Glycemia During Physical Activity (n=112, bouts=447)

	TIR			TBR			TAR		
Category of Protein Intake	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**
				Unadjusted Models					
≤ 10 grams protein (bouts=99)	0.80%	0.90	(-11.80%, 13.39%)	-1.06%	0.62	(-5.33%, 3.21%)	0.26%	0.97	(-12.82%, 13.34%)
10 - 19.9 grams protein (bouts=122)	-1.45%	0.81	(-13.59%, 10.69%)	**-5.08%**	**0.02**	**(-9.19%, -0.97%)**	6.15%	0.34	(-6.47%, 18.76%)
≥ 20 grams protein (bouts=159)	2.36%	0.69	(-9.30%, 14.02%)	**-5.86%**	**<0.01**	**(-9.80%, -1.91%)**	3.56%	0.56	(-8.56%, 15.67%)
				Fully Adjusted Models*					
≤ 10 grams protein (bouts=99)	2.58%	0.69	(-10.22%, 15.39%)	-0.46%	0.33	(-6.84%, 2.30%)	-2.03%	0.76	(-15.30%, 11.23%)
10 - 19.9 grams protein (bouts=122)	0.22%	0.97	(-12.10%, 12.54%)	**-4.41%**	**0.04**	**(-8.57%, -0.25%)**	3.85%	0.55	(-8.93%, 16.62%)
≥ 20 grams protein (bouts=159)	1.19%	0.85	(-11.17%, 13.54%)	**-4.83%**	**0.02**	**(-9.00%, -0.66%)**	3.74%	0.56	(-9.07%, 16.53%)

*Estimates are adjusted for Estimates were adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/mins, pre-exercise carbohydrate intake, and hours until midnight.
Reference Group = No Protein Intake Within 4 Hours Prior to MVPA Bouts (bouts=67)

Table 3.3. Results of Mixed Effects Regression Models Estimating the Association Between Relative Protein Intake (grams/kg) Consumed Within 4 Hours Prior to a Bout of Moderate-to-Vigorous Physical Activity and Glycemia During Physical Activity (n=112, bouts=447)

	TIR			TBR			TAR		
Category of Protein Intake	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**
				Unadjusted Models					
<12.5grams/kg (bouts=72)	4.33%	0.53	(-9.15%, 17.81%)	0.91%	0.69	(-3.63%, 5.45%)	-5.28%	0.46	(-19.23%, 8.67%)
0.125 - 0.249grams/kg (bouts=102)	-0.90%	0.89	(-13.45%, 11.65%)	**-5.69%**	**0.01**	**(-9.92%, -1.47%)**	6.57%	0.32	(-6.42%, 19.58%)
≥0.25grams/kg (bouts=206)	0.12%	0.98	(-11.14%, 11.38%)	**-5.56%**	**<0.01**	**(-9.35%, -1.77%)**	5.35%	0.37	(-6.32%, 17.02%)
				Fully Adjusted Models*					
<12.5grams/kg (bouts=72)	6.27%	0.37	(-7.41%, 19.95%)	1.60%	0.49	(-2.98%, 6.19%)	-7.80%	0.28	(-21.93%, 6.33%)
0.125 - 0.249grams/kg (bouts=102)	0.70%	0.91	(-11.98%, 13.38%)	**-5.38%**	**0.01**	**(-9.63%, -1.13%)**	4.72%	0.48	(-8.39%, 17.82%)
≥0.25grams/kg (bouts=206)	-0.71%	0.91	(-12.48%, 11.07%)	**-4.32%**	**0.03**	**(-8.27%, -0.38%)**	4.94%	0.42	(-7.23%, 17.10%)

*Estimates are adjusted for Estimates were adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/mins, pre-exercise carbohydrate intake, and hours until midnight.
Reference Group = No Protein Intake Within 4 Hours Prior to MVPA Bouts (bouts=67)

Table 3.4. Results of Mixed Effects Regression Models Estimating the Association Between Protein Intake (grams) Within 4 Hours Prior to a Bout of Moderate-to-Vigorous Physical Activity and Glycemia Following Cessation of Physical Activity until 6:30 AM the Following Morning (n=112, bouts=447)

	TIR			TBR			TAR		
Category of Protein Intake	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**
				Unadjusted Models					
≤ 10 grams protein (bouts=99)	1.20%	0.74	(-6.00%, 8.39%)	-0.29%	0.81	(-2.68%, 2.10%)	-0.71%	0.85	(-8.30%, 6.89%)
10 - 19.9 grams protein (bouts=122)	1.04%	0.77	(-5.90%,7.98%)	-1.13%	0.33	(-3.44%, 1.17%)	0.30%	0.94	(-7.03%, 7.63%)
≥ 20 grams protein (bouts=159)	1.45%	0.67	(5.22%, 8.12%)	0.28%	0.8	(-1.93%, 2.49%)	-1.41%	0.69	(-8.45%, 5.63%)
				Fully Adjusted Models*					
≤ 10 grams protein (bouts=99)	1.56%	0.67	(-5.75%, 8.87%)	-0.50%	0.68	(-2.92%, 1.93%)	-0.92%	0.81	(-8.64%, 6.80%)
10 - 19.9 grams protein (bouts=122)	1.72%	0.63	(-5.31%, 8.74%)	-1.16%	0.33	(-3.49%, 1.17%)	-0.37%	0.92	(-7.79%, 7.06%)
≥ 20 grams protein (bouts=159)	0.54%	0.88	(-6.32%, 7.40%)	0.30%	0.80	(-1.98%, 2.57%)	-0.52%	0.89	(-7.76%, 6.72%)

*Estimates are adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/mins, daily carbohydrate intake, post-activity protein intake and hours until midnight.
Reference Group = No Protein Intake Within 4 Hours Prior to MVPA Bouts (bouts=67)

Table 3.5. Results of Mixed Effects Regression Models Estimating the Association Between Relative Protein Intake (grams/kg) Within 4 Hours Prior to a Bout of Moderate-to-Vigorous Physical Activity and Glycemia Following Cessation of Physical Activity until 6:30 AM the Following Morning (n=112, bouts=447)

	TIR			TBR			TAR		
Category of Protein Intake	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**	**Estimate**	**P-Value**	**95% CI**
				Unadjusted Models					
<12.5grams/kg (bouts=72)	0.91%	0.81	(-6.76%, 8.59%)	-0.69%	0.59	(-3.25%, 1.86%)	0.02%	1.00	(-8.08%, 8.12%)
0.125 - 0.249grams/kg (bouts=102)	-0.85%	0.81	(-8.01%, 6.32%)	-0.22%	0.86	(-2.61%, 2.17%)	1.27%	0.74	(-6.30%, 8.83%)
≥0.25grams/kg (bouts=206)	2.49%	0.45	(-3.95%, 8.93%)	-0.24%	0.83	(-2.38%, 1.91%)	-1.97%	0.57	(-8.78%, 4.83%)
				Fully Adjusted Models*					
<12.5grams/kg (bouts=72)	1.11%	0.78	(-6.69%, 8.91%)	-0.86%	0.51	(-3.46%, 1.73%)	-0.05%	0.99	(-8.29%, 8.18%)
0.125 - 0.249grams/kg (bouts=102)	-0.24%	0.95	(-7.47%, 7.00%)	-0.07%	0.96	(-2.47%, 2.34%)	0.50%	0.9	(-7.14%, 8.14%)
≥0.25grams/kg (bouts=206)	2.13%	0.52	(-4.48%, 8.74%)	-0.42%	0.71	(-2.62%, 1.78%)	-1.47%	0.68	(-8.45%, 5.51%)

*Estimates are adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/mins, daily carbohydrate intake, post-activity protein intake and hours until midnight.
Reference Group = No Protein Intake Within 4 Hours Prior to MVPA Bouts (bouts=67)

3.4. DISCUSSION

This study utilized existing data from the FLEX trial to explore a unique intersection between diabetes care and sports nutrition by evaluating the role of pre-exercise protein intake on glycemia during and following exercise among adolescents with T1D. It was hypothesized that elevated protein intake within the 4 hours prior to MVPA bouts would be associated with improved TIR and reduced TBR during and following exercise. The results of this study demonstrated that consumption of at least 10g or 0.125g/kg bodyweight was associated with reduced TBR during MVPA, indicating improved safety for adolescents with T1D. No association was observed between pre-exercise protein intake and TIR or TAR during exercise. Similarly, no association was observed between pre-exercise protein intake and glycemia following exercise.

These findings are in agreement with the findings of Dube et al. who observed that, among adolescents with T1D, consuming a protein supplemented breakfast two hours prior to exercise was equally effective at preventing hypoglycemia during exercise compared to a standard breakfast that was followed by consumption of a carbohydrate beverage 15 minutes prior to exercise.[26] While the size of the reduction in TBR may appear relatively small (4.32% – 5.69% or ~ 2.59 – 3.41 minutes), it is important to note that the mean TBR during physical activity among participants in this study was 3.13% ± 14.0% and guidelines recommend that TBR be minimized among adolescents with T1D.[27] As such these findings represent a clinically significant decrease in TBR during physical activity. While previous studies have shown that the hyperglycemic effect of protein intake among adolescents with T1D may persist for 5 hours or longer, [23,24,134] we did not observe an association between pre-exercise protein intake and glycemia following that MVPA. It is also important to note that, in healthy populations,

consuming protein prior to exercise has been suggested to have potential benefits for promoting recovery or reducing fatigue during exercise among healthy populations.[123,129,131,152] While such results effects haven't been tested among people with T1D, it's possible that consuming protein prior to exercise may be a promising strategy to assist people with T1D in improving both the safety and benefits of exercise.

Challenges and Opportunities

As with all studies, this study has several limitations. First, self-reported measures of dietary intake are prone to under-reporting due to recall and social desirability biases [153], however, the use of a multiple pass method for 24-hours dietary intake data, as was used in the FLEX study, has been shown to minimize the effects of these biases in dietary intake data. [62,154] Furthermore, MVPA is often over-reported among adolescents compared to accelerometry [155] which may have influenced the number of bouts that we identified. The PDPAR instrument that we utilized in the FLEX study, however, has been validated among adolescents against accelerometers for both relative energy expenditure ($r=0.77$, $p<0.01$) and identification of MVPA bouts on a previous day (0.63, $p<0.01$). [147,156] Furthermore, the use of interviewers to administer recalls of physical activity has been shown provide more reliable measurement of MVPA compared to self-administered methods. [157] Additionally, the lack of time-stamped insulin dosing data for these analyses limits are ability to understand the role of insulin-dosing behaviours on the observed associations. By controlling for carbohydrate intake in these analyses we hoped to partially account for bolus insulin levels, which are determined by carbohydrate intake, however, we cannot account for basal insulin dosing and potential insulin dosing strategies which may have been implemented to reduce the risk of exercise-related hypoglycemia.

Finally, approximately 26% of identified bouts of MVPA were missing adequate CGM data which may be a source of selection bias in our analyses. In exploration of differences between those with and without adequate CGM data we did not observe any significant differences between the groups by any variable included in our analyses which may indicate the amount of selection bias present in these analyses is minimal. The availability of time-stamped CGM, dietary intake and physical activity measures, however, provided a unique opportunity to observe a temporal relationship between pre-exercise protein intake and glycemia during and following physical activity, which begin to address an important gap in the literature and start bridging sports nutrition and diabetes care guidelines.

Future Directions

Randomized controlled trials are needed to establish whether a causal relationship exists between pre-exercise protein intake and glycemia during exercise and the hours thereafter among adolescents and adults with T1D. As fear of hypoglycemia is major barrier to regular participation in physical activity among people with T1D, these trials are essential to continuing to address these important gaps in our understanding of the role of peri-exercise protein intake on exercise-related glycemia and to inform dietary guidelines to support safe participation in exercise for those living with T1D. Additionally, while safe participation in exercise is the primary concern for people living with T1D, there are numerous reasons for which a person may decide to participate in exercise that we should aim to support when forming nutritional guidelines. As such, future work should continue to strive to bridge sports nutrition and diabetes care guidelines to help identify nutritional strategies which may promote both enhanced glycemic management and positive adaptive benefits with exercise among people living with T1D.

CHAPTER 4. A HIGH PROTEIN DIET IS ASSOCIATED WITH IMPROVED GLYCEMIC CONTROL FOLLOWING EXERCISE AMONG ADOLESCENTS WITH TYPE 1 DIABETES

4.1. Introduction

Type 1 diabetes (T1D) is one of the leading causes of chronic disease in youth, with an estimated prevalence of 9 million people globally.[48] In 2017, the estimated prevalence of T1D among youth in the United States was 2.15 per 1000 youth, which represents a relative increase of 45.1% since 2001.[50] Type 1 diabetes is associated with numerous health complications including a risk of cardiovascular disease approximately ten times that of those without diabetes.[2] In the Diabetes Complications and Control Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC), the extensive health benefits of intensive insulin therapy were highlighted, reporting a significant 57% and 42% reduction in cardiovascular disease events and mortality, respectively, among people with T1D. This same study, however, also observed that intensive insulin therapy was associated with weight gain and, among those classified as excessive weight gainers, the benefits of intensive insulin therapy were substantially diminished, with no difference in cardiovascular disease risk or mortality between those on intensive insulin therapy who gained excessive weight and those on conventional therapy at 6 years follow-up.[65,158]

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1. Muntis FR, Smith-Ryan AE, Crandell J, et al. A High Protein Diet Is Associated with Improved Glycemic Control Following Exercise among Adolescents with Type 1 Diabetes. Nutrients. 2023;15(8).

Participation in regular physical activity is a central part of both diabetes and weight management for adolescents with T1D. The American Diabetes Association recommends that adolescents with T1D participate in at least 60 minutes/day of moderate-to-vigorous physical activity (MVPA).[66] Systematic reviews of physical activity and exercise interventions among youth with T1D indicate that regular physical activity is associated with improved glycemia, cardiorespiratory fitness, metabolic health, and weight management.[71,76,77] Despite these benefits of regular physical activity, research has shown that adolescents with T1D engage in lower levels of physical activity compared to their peers without diabetes, with as few as 37.8% achieving the World Health Organization (WHO) recommendations of at least 60 minutes of MVPA per day.[67,69,72] A major barrier to physical activity among adolescents with T1D is fear associated with the risk of experiencing hypoglycemia during and up to 24 hours following exercise.[5,16,143] Of particular concern is an increased risk of hypoglycemia overnight, which may often result in more severe episodes of hypoglycemia or diabetic ketoacidosis.[80-82] Dietary guidelines, particularly nutrient timing recommendations, are needed to help guide safe participation in physical activity for adolescents with T1D.

While expert recommendations for carbohydrate intake before or after exercise for people with T1D have been published[19], less is known regarding the effects of protein intake on glycemia following physical activity. Sports nutrition guidelines recommend consumption of 0.25-0.3g/kg or an absolute dose of 20-40g of protein following exercise, as well as the consumption of high protein meals every 3-4 hours following exercise to support recovery from and adaptation to an exercise bout.[116,121] Furthermore, in active individuals a higher protein diet (25-30% energy from protein) combined with regular exercise have been associated with improved muscular strength and reduced soreness and, significant decrease in fat mass when

paired with a caloric deficit.[113,120,159] It is possible that similar protein recommendations may also improve glycemia following exercise for those with T1D, although minimal data exists in this area.

Among adolescents with T1D, protein intake has been associated with a mild hyperglycemic effect which persists for at least 5 hours post prandial,[23,24,160] with one study suggesting that this effect may persist as long as 12 hours following larger meals.[134] Only two studies to the authors knowledge, have investigated the effects of protein intake on glycemia during or following exercise. One randomized controlled trial compared the effects of three different dietary approaches on glycemia during moderate intensity cycling exercise among adolescents with T1D (n=10): 1) a high protein breakfast (consumed two hours prior to exercise) plus a non-caloric placebo-beverage (consumed 15 minutes prior to exercise), 2) a standard breakfast plus a carbohydrate beverage, 3) a standard breakfast plus a non-caloric placebo beverage.[26] The authors demonstrated that, while the carbohydrate beverage approach showed the slowest decline in glycemia during exercise, the protein-supplemented breakfast was equally effective at preventing hypoglycemia.[26] Additionally, a recent laboratory-based pilot study found that, among 6 participants with T1D with a mean age 20.2 ± 3.1 years a 50g protein whey protein bolus compared to water, provided 3.25 hours after 45 minutes of moderate exercise significantly reduced the glucose required to maintain euglycemia overnight.[141] While these studies support the theory that peri-exercise protein intake may reduce the risk of exercise-related hypoglycemia, the samples for these studies were small and the highly controlled nature of the study designs may limit understanding of the efficacy of this nutritional strategy in a free-living environment.

As such, the primary aims of this study were to conduct secondary data analysis using data from a randomized trial of an adaptive behavioral intervention among adolescents with T1D to investigate the role of post-exercise (Aim 1) and daily protein intake (Aim 2) on glycemia following bouts of MVPA until the following morning. It was hypothesized that both post-exercise and daily protein intake would be associated with improvements in TIR and reductions in TBR following exercise among adolescents with T1D.

4.2. Materials and Methods

4.2.1. Study Design

To assess the proposed aims, post-hoc analyses were performed using data from a randomized controlled trial of an adaptive behavioral intervention among adolescents with T1D named the Flexible Lifestyles Empowering Change (FLEX) study (1UC4DK101132-01). The FLEX study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by institutional review boards at clinical sites in Colorado and Ohio, as well as the coordinating site at the University of North Carolina at Chapel Hill (IRB #13-2856, Approved 10/3/2013). The FLEX study enrolled 258 adolescents with T1D between the ages of 13-16 years from 05/01/2014 to 04/04/2016. These participants were randomized to receive either usual care (n=128) or an 18-month adaptive behavioral intervention (n=130) aimed at improving diabetes self-management skills. The intervention utilized motivational interviewing and problem-solving skills training to help participants identify strategies to improve glucose control. While the intervention incorporated behavioral strategies around self-management skills, including insulin dosing, blood glucose testing, diet, and physical activity, the intervention did not systematically incorporate guidance for increasing physical activity. Written assent was provided by study participants and written informed consent was provided by study participants' parents. These

post-hoc analyses utilize secondary measures from baseline and 6-months post-baseline visit to evaluate the proposed aims. Full details of the design and main results of the FLEX study have been published elsewhere.[144,145]

4.2.2. Participants

The FLEX study recruited participants from two clinical sites: the Barbara Davis Center for Childhood Diabetes in Colorado and Cincinnati Children's Hospital Medical Center in Ohio, with the University of North Carolina at Chapel Hill serving as a coordinating center from 05/01/2014 – 04/04/2016. Eligible criteria for the study included being between the ages of 13-16 years of age at study entry with a hemoglobin A1c (HbA1c) of 8-13% and a diabetes duration of greater than one year. Youth who were pregnant or had a severe concurrent physical, developmental, or psychiatric medical conditions were excluded from participating in the study. For the secondary analyses reported in this study, participants were included if they had reported a bout of MVPA at baseline or 6-months post-baseline visit and had sufficient dietary and glycemia data on the same day as the reported physical activity. Baseline demographic, clinical, glycemia, dietary, and physical characteristics among participants included in our analyses (n=114) were evaluated and are reported in Table 1. Continuous variables are reported as mean and standard deviation except for non-normally distributed variables, in which median and interquartile range were reported. Categorical variables are described with counts and percentages.

4.2.3. Measures

Demographics and Health History

Demographic questionnaires were completed at baseline from which self-reported age, sex, race/ethnicity were reported. Health history questionnaires were completed at baseline, from which participants reported their date of diabetes diagnosis, insulin regimen, and total previous day insulin dose, among other measures. Follow-up health history questionnaires were administered 6- and 18-months post-intervention to report any changes in health history or diabetes care since their baseline visit. Age, sex, race/ethnicity, diabetes duration, insulin regimen, previous day insulin dose, and previous day insulin dose per kilogram of bodyweight were considered as potential covariates in our statistical models.

Continuous Glucose Monitoring (CGM)

Participants in the FLEX study were asked to wear a blinded Medtronic iPro2 continuous glucose monitor with an Enlite sensor for 7-days at baseline, 6 months, and 18 months post-intervention. As dietary and physical activity data were collected at baseline and 6 months, but not 18 months, CGM data from the 18 month visit are not included in these analyses. To enhance compliance and improve the quality of CGM data collection, an iPro2 compatible meter (OneTouch Ultra2) was provided to the participant along with 50 test strips for calibration 1- and 3-hours after insertion, pre-meal and before bed. Utilizing consensus report definitions[27], our outcomes of percent time in range (TIR, 70-180mg/dL), percent time above range (TAR, >180mg/dL), and percent time below range (TBR, <70mg/dL) were calculated from the cessation of a bout of MVPA until 6:30AM the following morning to prevent confounding by dietary intake the following day. As the hyperglycemic effect of protein is known to last at least

5 hours, observations with fewer than 5 hours of CGM data following activity were excluded from our analyses.

Dietary Measures

During the 7-day CGM wear time, two unannounced 24-hour dietary recalls were collected at baseline and 6-months post-intervention by certified interviewers from the UNC NIH/NIDDK Nutrition Obesity Research Center (NORC) staff (P30DK056350, MPI Mayer-Davis, Shaikh), using the Nutrient Data System for Research software and the multiple pass interviewing method.[62,150] For these analyses, participants with relative daily protein intakes greater than 3 standard deviations above the mean (>3.21g/kg) where excluded as potential outliers. Our Aim 1 exposure of post-exercise protein intake was defined as protein intake consumed between the end of a bout of MVPA and the end of the day (midnight) in both grams & grams/kg of bodyweight. Furthermore, as sports nutrition guidelines recommend daily protein intakes of 1.2-2.0g/kg bodyweight to promote positive physiological adaptation to exercise[116], we further explored the effect of daily protein intake levels on glycemia for our Aim 2 analyses by comparing CGM metrics of TIR, TAR, and TBR from the cessation of MVPA bouts until the following morning between those who consumed < 1.2g/kg bodyweight and those who consumed ≥1.2g per kilogram bodyweight.

Physical Activity Measures

At baseline and 6 months following the baseline visit, two previous day physical activity records (PDPAR) were collected during the 7-day CGM wear time by certified interviewers in conjunction with the 24-hour dietary recalls. The PDPAR is a validated questionnaire which asks participants to describe the dominant activity and approximate intensity of activities they

performed during the previous day in 30-minute time blocks.[147] Intensities are described in categories as very light (slow breathing with little or no movement), light (normal breathing with regular movement), medium (increased breathing and quick movement for short periods of time), or hard (hard breathing with quick movement for ≥20 minutes). Each activity and perception of effort are matched to a corresponding metabolic equivalent (MET) value.[147,148] From these records, bouts of MVPA were defined as 30 minutes or greater of physical activity at a MET of greater than or equal to 3 METs. Average intensity (METs), bout duration (minutes) and bout volume (MET-minutes) were considered as potential covariates in our statistical models.

Anthropometrics and Body Composition

Height, weight, and natural waist circumference were measured utilizing a wall-mounted stadiometer, calibrated electric scale, and a flexible fiberglass or steel tape measure, respectively, at baseline, 6-, and 18-months post-intervention. From these measures, body fat percentage was estimated using validated age, race, and gender specific equations.[151] Estimated body fat percentage was considered as a potential covariate in our statistical models.

4.2.4. Statistical Analysis

Model Selection

All statistical analyses were performed using SAS 9.4 (Cary, NC). To account for repeated measures, mixed effects regressions models were utilized for both our Aim 1 and Aim 2 analyses utilizing the Proc Mixed command. Potential covariates were introduced into our models in groups of design (study site, intervention group) demographic (age, sex, race/ethnicity), clinical (diabetes duration, insulin regimen, total previous day insulin dose, previous day insulin dose per kilogram bodyweight), body composition (estimated body fat

percentage), physical activity (average bout intensity (METs), bout duration (minutes), bout volume (MET-minutes), other daily physical activity (MET -minutes)), dietary (daily carbohydrate intake, pre-exercise protein intake) and timing variables (hours until midnight). Covariates that caused a ≥10% change in the point estimate or standard error of associations were included in our final models.

Aim 1 Analyses – Post-exercise Protein Intake & Glycemia Following MVPA

Figure 1 provides an illustrated example timeline of exposures and outcomes relative to a bout of MVPA for both our Aim 1 and Aim 2 analyses. Post-exercise protein intake was defined continuously as protein intake (grams & grams/kg) from the cessation of a bout of MVPA until midnight. Mixed effects regression models assessed the association between post-exercise protein intake and TIR, TBR, and TAR from the cessation of a bout of MVPA until 6:30am the following morning. Final models adjusted for intervention group, study site, age, sex, race/ethnicity, diabetes duration, insulin regimen, estimated body fat percentage, MVPA bout volume (MET-minutes), other daily MVPA (MET-mins), daily carbohydrate intake, protein intake consumed within 4 hours prior to exercise, and hours until midnight.

Aim 2 Analyses – Overall Daily Protein Intake & Glycemia Following MVPA

To align with sports nutrition guidelines which recommend intakes of 1.2 – 2.0g/kg/day of dietary protein to support exercise training, observations were categorized by daily protein intake into those with <1.2g/kg/day of protein and those with ≥ 1.2g/kg/day of protein utilizing a binomial variable. The relationship between protein intake category and CGM metrics of TIR, TBR, and TAR were assessed, with observations classified as <1.2g/kg chosen as the reference group. The final analytic model adjusted for intervention group, study site, age, sex,

race/ethnicity, diabetes duration, insulin regimen, estimated body fat percentage, bout volume (MET-minutes), other daily MVPA (MET-mins), daily carbohydrate intake, and hours until midnight.

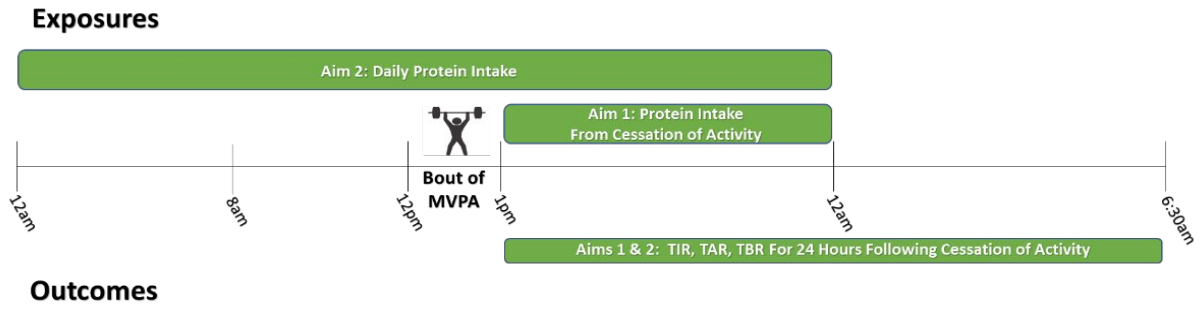


Figure 4.1. Example Timeline of Exposures & Outcomes Relative to a Bout of Moderate-to-Vigorous Physical Activity Using Multiple Measurements (Baseline & 6 Months). Individuals may report multiple bouts of moderate-to-vigorous physical activity (MVPA) per day.

4.2.9. Exploration of Interaction Effects

Interaction effects were explored by sex, weight status, insulin regimen, MVPA bout volume (MET-mins), and whether a bout was vigorous (average bout MET-value ≥ 6.0) or moderate (average bout MET-value <6.0). The decision to include these terms was based off of previous studies which have indicated that, among adolescents with T1D, those who utilize multiple daily insulin injections, those who have overweight or obesity, those with higher physical activity loads and also female adolescents may experience more difficulties in managing glycemia which may then influence the association of protein intake on post-exercise glycemia in a free-living environment.[161-166] Interaction terms were added to the final Aim 1 & Aim 2 mixed effects regression models to assess for potential differences in the response to post-exercise protein intake or daily protein intake category on glycemic metrics from the end of a MVPA bout until the following morning. Tanner score was also added as a covariate in statistical models assessing for differences by sex. Weight status was defined using BMI z-score to categorize participants by whether they had overweight/obesity or not at the time of their most

recent study visit. Statistical significance for interaction effects were determined at a p-value <0.10.

4.3. RESULTS

Final Sample Size

Of the 258 participants in the FLEX study, 135 participants reported at least 1 MVPA bout with a total of 645 MVPA bouts identified. From these 162 bouts reported, 56 participants had insufficient or missing CGM data. Additionally, 7 bouts reported from 1 participant were excluded for reporting protein intakes above 3 standard deviations about the mean (>3.21g/kg/day). Furthermore, 11 bouts (n=5) were excluded for missing data on weight, 10 bouts (n=1) were excluded for missing insulin regimen data, and 1 bout (n=1) was excluded for missing sufficient data to estimate body fat percentage. As participants may have reported multiple bouts at both baseline and 6-month study visits, exclusion of a bout does not necessarily indicate full exclusion of a participant. Our final analytic models included 454 bouts from 114 participants as detailed in **Figure 4.2**. In sensitivity analyses, we explored differences between FLEX participants included in our analyses and those not included (n=114 vs. n=144) in regard to the baseline characteristics provided in Table 1 and no significant differences were observed. Additionally, in exploration of potential differences between bouts that were included versus those excluded for insufficient or missing CGM data (bouts=454 vs bouts=162), no significant differences were observed for post-exercise or daily protein, carbohydrate, fat or calorie intake, any demographic variables included in Table 1, or any other variable included in our analytic models.

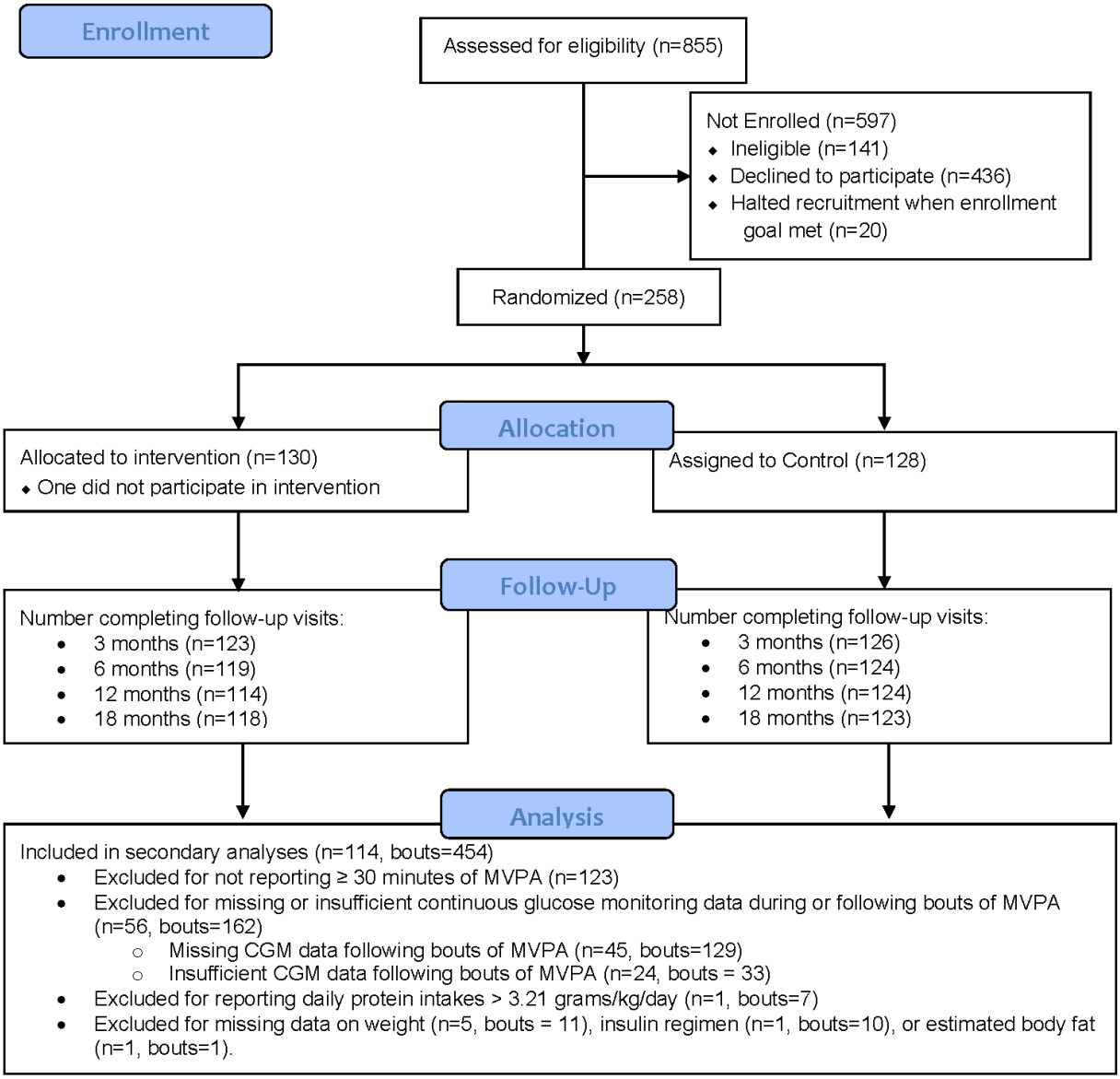


Figure 4.2. Consort Diagram for Secondary Analyses of the Flexible Lifestyles Empowering Change (FLEX) Randomized Trial

Baseline Characteristics

Baseline characteristics of FLEX participants included in our analyses are provided in **Table 4.1**. The median age, diabetes duration, and estimated body fat percentage for participants at baseline was 14.5 (IQR: 13.8, 15.7), 5.4 (IQR: 3.1, 9.0), and 28.1% (20.1%, 33.1%), respectively. There was similar inclusion of male (46.0%) and female (54.0%) participants. Most participants reported meeting WHO guidelines of achieving at least 60 minutes MVPA per day (97.3%). Furthermore, while the majority of participants (72.3%) reported using insulin pumps in their diabetes care, a majority of participants (69.9%) reported not using a continuous glucose monitor in their diabetes care in the past 30 days. Additionally, at baseline, participants had a median HbA1c of 9.3% (8.6%, 9.9%) and spent 36.4% ± 13.7% TIR, 59.7% ± 16.0% TAR, and 2.1% (IQR: 0.3%, 5.6%) TBR during their 7-day CGM wear time.

Table 4.1: Baseline Characteristics of FLEX Participants Included in Final Analyses (n=114)

Demographic		Mean ± SD or N (%)
Age		14.5 (13.8, 15.7)
Female		61 (54.0)
Male		52 (46.0)
Race/Ethnicity		
	Non-Hispanic White	91 (80.5)
	Non-Hispanic Black	2 (1.8)
	Hispanic	14 (12.4)
	Multiracial/Other	6 (5.3)
Maximum Education of Parents		
	High School or Less	11 (9.8)
	Some College	31 (27.7)
	Four Year College Degree	50 (44.6)
	Graduate Degree	20 (17.9)
Clinical		
Diabetes Duration		5.4 (3.1, 9.0)
Insulin Pump User (n=111)		81 (72.3)
Previous Day Insulin Dose (units/kg) (n=110)		1.0 ± 0.3
Anthropometric		
Weight (kg)		58.8 (51.3, 69.2)
BMI Z-Score		0.7 ± 0.9
Estimated Body Fat %		28.1 (20.1, 33.1)
Glycemia		
No Personal CGM Use in Past 30 Days (n=103)		72 (69.9)
Baseline HbA1c (%)		9.3 (8.6, 9.9)
Percent Time in Range (n=106)		36.4 ± 13.7
Percent Time Below Range (n=106)		2.1 (0.3, 5.6)
Percent Time Above Range (n=106)		59.7 ± 16.0
Diet		
Daily Caloric Intake (kcal)		1623.3 (1315.6, 2062.0)
Percent of Daily Calories from Protein		16.0 ± 3.5
Percent of Daily Calories from Carbohydrate		49.0 ± 7.7
Percent of Daily Calories from Fat		36.2 ± 6.4
Daily Fiber Intake (grams)		13.4 (10.2, 18.2)
Physical Activity (n = 109)		
Meet WHO Guidelines of ≥60mins MVPA/day		101 (92.7)
Daily Minutes of MVPA		165.0 (105.0, 225.0)
Daily Minutes of Vigorous Physical Activity		45.0 (0.0, 90.0)

Continuous variables are reported as mean and standard deviation except for non-normally distributed variables, in which median and interquartile range are reported. Categorical variables are described with counts and percentages.

Aim 1 Results

The median bout duration (minutes) and intensity (METs) were 60 (IQR: 30, 90) and 4.5 (IQR: 4.0, 7.0), respectively. The average time from the cessation of MVPA bouts until midnight and 6:30 AM the following morning was 9.0 ± 3.9 and 15.5 ± 10.4 hours, respectively. The median protein intake from MVPA bout cessation until midnight was 34.9 (IQR: 20.9, 52.7) grams or 5.6 (IQR: 31.1, 0.86) grams/kilogram of bodyweight. The median TIR, TAR, & TBR from MVPA bout cessation until the following morning were 40.6% (IQR: 21.6%, 59.7%), 56.4% (IQR: 35.4%, 75.7%), and 0.00% (0.00%, 3.2%), respectively.

We observed no association between post-exercise protein intake and TAR, TIR, or TBR when examined in grams or grams per kilogram **(Table 4.2).** Additionally, we observed no statistically significant interaction effects between post-exercise protein intake and MVPA bout volume (p-values > 0.36), insulin regimen (p-values > 0.24), or weight status (p-values > 0.27) for TAR, TIR or TBR. We did, however, observe a significant interaction between post-exercise protein intake in grams per kilogram (interaction p=0.03), but not grams (interaction p=0.16) with sex for TBR, indicating a borderline significant association of -1.4% (95% CI: -1.7%, 0.0%) TBR per 0.25g/kg protein among female participants, but not male participants, -0.1% (95% CI: -0.5%, 0.7%). Additionally, we observed significant interaction effects between post-exercise protein intake and sex when examined in grams (interaction p=0.02) and grams per kilogram (interaction p=0.03) with TIR of 3.6% (95% CI: 0.4%, 6.8%) per 20g and 2.3% (95% CI: -0.1%, 4.7%) per 0.25g/kg among females, but no association among males, -0.4% (-2.3%, 1.4%) per 20g and 0.3% (95% CI: -2.7%, 2.0%) per 0.25g/kg. We did not observe a significant interaction effect between post-exercise protein intake in grams (interaction p=0.10) or grams per kilogram

(p=0.19) with sex for TAR. Differences in association between post-exercise protein and glycemia following exercise by sex are depicted in **Figure 4.3.**

Table 4.2. Results of Mixed Effects Regression Models Assessing the Association Between Post-Exercise Protein Intake and Glycemia Following a Bout of MVPA Until 6:30AM The Following Morning Among Adolescents With Type 1 Diabetes (n=114, bouts=454)

	Post-Exercise Protein (grams)*			**Post-Exercise Protein (g/kg)†**		
	Estimate	**p-value**	**95% CI**	**Estimate**	**p-value**	**95% CI**
			Unadjusted Models			
Time Above Range	0.5%	0.52	(-1.1%, 2.2%)	0.6%	0.33	(-0.6%, 1.9%)
Time In Range	-0.4%	0.58	(-2.0%, 1.1%)	-0.6%	0.35	(-1.7%, 0.6%)
Time Below Range	0.1%	0.63	(-0.6%, 0.4%)	-0.1%	0.77	(-0.4%, 0.3%)
			Fully Adjusted Models‡			
Time Above Range	-0.7%	0.56	(-3.0%, 1.6%)	-0.1%	0.93	(-1.8%, 1.6%)
Time In Range	0.8%	0.49	(-1.4%, 2.9%)	0.2%	0.31	(-1.4%, 1.8%)
Time Below Range	0.1%	0.79	(-0.8%, 0.6%)	-0.1%	0.66	(-0.7%, 0.4%)

*Associations are reported per a 20g dose of protein
†Associations are reported per a 0.25g/kg dose of protein
‡Models are adjusted for design (study site, intervention group), demographic (age, sex, race/ethnicity), clinical (diabetes duration, insulin regimen), anthropometric (estimated body fat percentage), dietary (daily carbohydrate intake and pre-exercise protein intake), physical activity (bout volume, other daily physical activity), and hours until midnight.

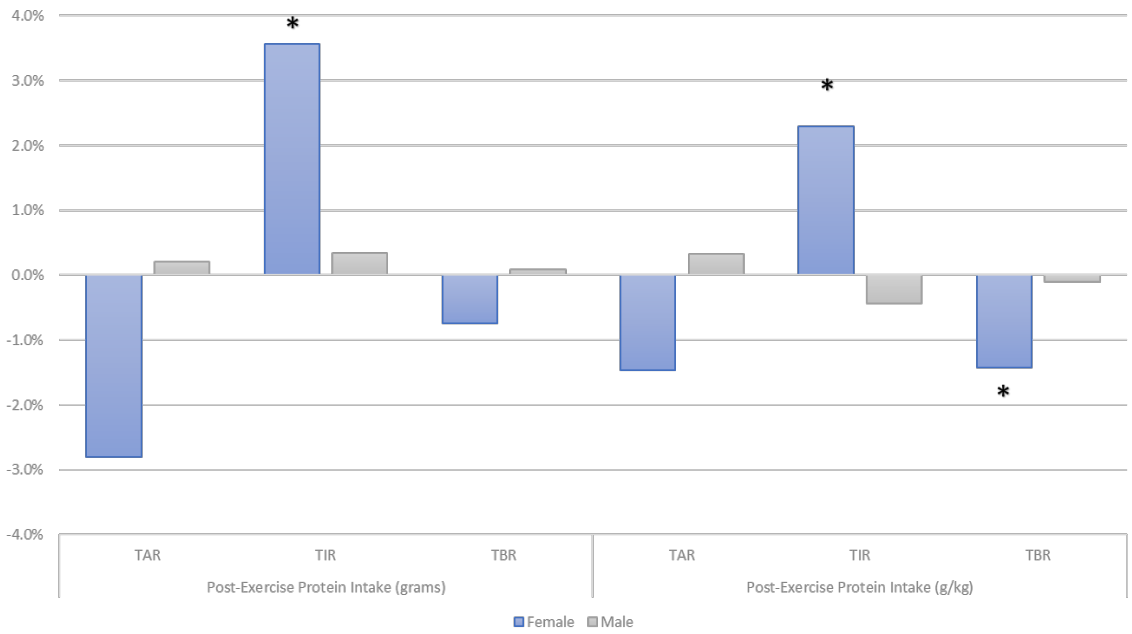


Figure 4.3. Differences in association between post-exercise protein intake and glycemia from cessation bouts of MVPA until the following morning by sex among adolescents with T1D. Estimates are provided per 20g or 0.25g/kg. TAR = percent time above range (>180mg/dL), TIR = percent time in recommended glucose range (70-180mg/dL), and TBR = percent time below range (<70mg/dL) following bouts of MVPA.

Aim 2 Results

The median daily protein intake reported on days with included bouts of MVPA was 65.4 (IQR: 48.2, 87.9) grams or 1.07 (IQR: 0.76, 1.48) grams/kg. Of the 454 bouts included in our final analyses, 188 bouts had daily protein intakes >1.2g/kg/day and 266 reported daily protein intakes below 1.2g/kg/day. Aim 2 results are provided in Table 3. Daily protein intakes of ≥1.2g/kg/day were associated with 8.0% (95% CI: 1.6%, 14.5%) less TAR and 6.9% (95% CI: 0.9%, 13.0%) greater TIR with no significant difference in TBR, 1.2% (95% CI: -0.8%, 3.2%). Additionally, we observed no significant interaction effect between daily protein intake category and MVPA bout volume (p-values > 0.56) or whether a bout was vigorous or moderate (p-values > 0.29) for TAR, TIR, TBR.

A significant interaction effect was observed between daily protein intake category and insulin regimen for TIR ($p=0.03$) and TAR ($p=0.08$), but not for TBR which indicated that adolescents who use multiple daily insulin injections (MDII) for their diabetes management may experience greater improvements in TIR, 17.9% (95% CI: 6.1%, 29.7%) and TAR, -17.9% (95% CI: -30.5%, -5.3%) (**Table 4.4**). Additionally, significant interaction effects were observed between protein intake category and weight status for TIR ($p<0.01$), TBR ($p<0.01$), and TAR ($p=0.08$) indicating that following a high protein diet pattern may improve TIR, 18.6% (95% CI: 8.7%, 28.4%), and TAR,-15.6% (95% CI: -26.1%, -5.1%) to a greater extent for adolescents with overweight/obesity, but may also elevate TBR, 2.7% (95% CI: 0.6%, 4.9%) among normal weight individuals. Following additional adjustment for tanner stage, significant interaction effects were also observed between protein intake category and sex for TIR ($p<0.01$) and TAR($p<0.01$) indicating that following a high protein diet pattern may improve TIR, 16.3% (95% CI: 8.4%, 24.2%), and TAR, -16.9% (-25.3%, -8.5%), to a greater extent among female adolescents. **Figure 4.4** shows a comparison of the effects of a high protein diet on post-exercise glycemia by insulin regimen, weight status, and sex.

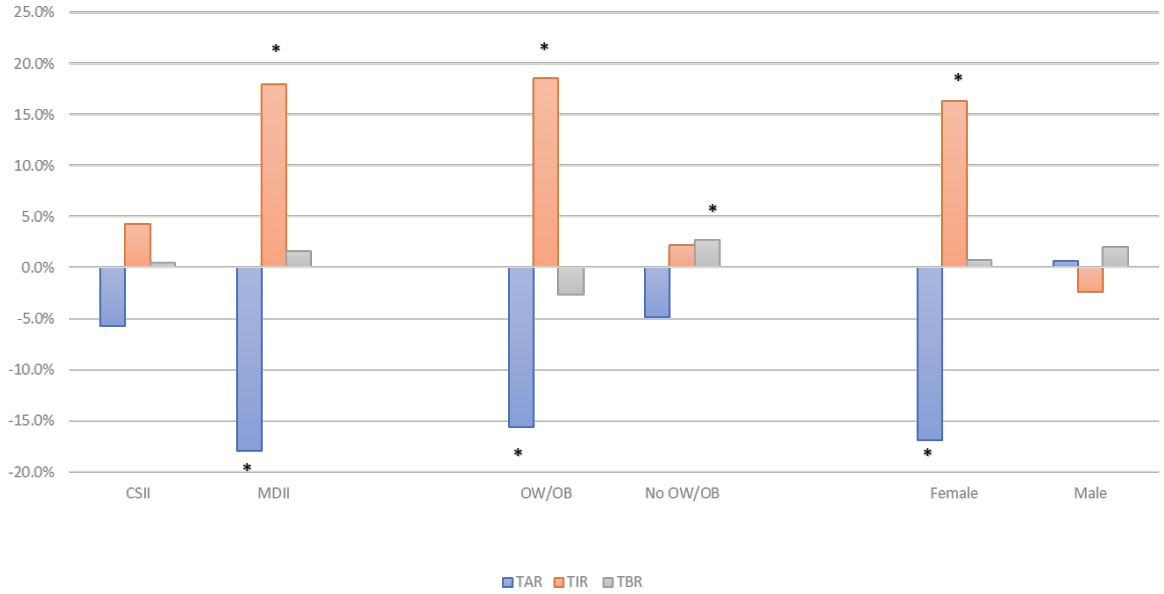


Figure 4.4. Differences in the effect of consuming a higher protein diet (>1.2g/kg/day) on glycemia from the end of MVPA bouts until the following morning by insulin regimen, weight status, and sex. CSII = Continuous Subcutaneous Insulin Infusion, MDII = Multiple Daily Insulin Injections, OW/OB = Overweight or Obesity, No OW/OB = no overweight or obesity. TAR = percent time above range (>180mg/dL), TIR = percent time in recommended glucose range (70-180mg/dL), TBR = percent time below range (<70mg/dL) following bouts of MVPA.

Table 4.3. Results of Linear Mixed Effects Regression Models Comparing Continuous Glucose Monitoring Metrics Following Cessation of Bouts of Moderate-to-Vigorous Physical Activity until 6:30 AM the Following Morning by Category of Daily Protein Intake (g/kg/day) Among adolescents With Type 1 Diabetes (n=114, bouts=454)

Category of Daily Protein Intake	% Time Above Range			% Time in Range			%Time Below Range		
	Estimate	p-value	95% CI	Estimate	p-value	95% CI	Estimate	p-value	95% CI
Unadjusted Models									
<1.2g/kg/day (bouts=266)	Reference								
>1.2g /kg/day (bouts=188)	-6.8%	0.02	(-12.4%, -1.1%)	5.3%	0.05	(0.0%, 10.6%)	1.5%	0.09	(-0.3%, 3.2%)
Fully Adjusted Models*									
<1.2g/kg/day (bouts=266)	Reference								
>1.2g/kg/day (bouts=188)	-8.0%	0.02	(-14.5%, -1.6%)	6.9%	0.03	(0.9%, 13.0%)	1.2%	0.22	(-0.8%, 3.2%)

*Final Models were adjusted for design (intervention group, study site), demographic (age, sex, race/ethnicity), clinical (diabetes duration, insulin regimen), anthropometric (estimated bodyfat percentage), physical activity (bout volume (MET-mins), other daily MVPA (MET-mins), dietary (daily carbohydrate intake), and timing (hours until midnight) variables.

Table 4.4. Results of mixed effects regression models assessing interaction between daily protein intake category with insulin regimen, weight status, and sex

Interaction Effects*	% Time Above Range			% Time in Range			% Time Below Range		
	Estimate	**p-value**	**95% CI**	**Estimate**	**p-value**	**95% CI**	**Estimate**	**p-value**	**95% CI**
Insulin Regimen	Interaction P-Value = 0.08			Interaction P-Value = 0.03			Interaction P-Value = 0.60		
Continuous Subcutaenous Insulin Infusion (CSII)	-5.7%	0.1	(-12.5%, 1.1%)	4.2%	0.19	(-2.2%, 10.6%)	0.5%	0.81	(-3.4%, 4.4%)
Multiple Daily Insulin Injections (MDII)	**-17.9%**	**<0.01**	**(-30.5%, -5.3%)**	**17.9%**	**<0.01**	**(6.1%, 29.7%)**	1.6%	0.13	(-0.55%, 3.8%)
Weight Status	Interaction P-Value = 0.08			Interaction P-Value <0.01			Interaction P-Value <0.01		
Overweight/Obesity	**-15.6%**	**<0.01**	**(-26.2%, -5.1%)**	**18.6%**	**<0.001**	**(8.7%, 28.4%)**	-2.6%	0.11	(-5.8%, 0.6%)
No Overweight/Obesity	-4.9%	0.18	(-12.0%, 2.3%)	2.2%	0.52	(-4.5%, 8.8%)	**2.7%**	**0.01**	**(0.6%, 4.9%)**
Sex	Interaction P-Value <0.01			Interaction P-Value <0.01			Interaction P-Value =0.48		
Female	**-16.9%**	**<0.001**	**(-25.3%, -8.5%)**	**16.3%**	**<0.001**	**(8.4%, 24.2%)**	0.7%	0.61	(-2.0%, 3.4%)
Male	0.6%	0.88	(-7.7%, 9.0%)	-2.4%	0.56	(-10.3%, 5.5%)	2.0%	0.14	(-0.7%, 4.7%)

*Interaction models estimated the combined effect of increasing protein intake category from <1.2g/kg/day to ≥1.2g/kg/day and category of insulin regimen (CSII/MDII), weight status (has overweight/obesity or does not have overweight/obesity) or sex. Mixed effects regression models were adjusted for design (intervention group, study site), demographic (age, sex, race/ethnicity), clinical (diabetes duration, insulin regimen), anthropometric (estimated bodyfat percentage (protein*Insulin regimen) or weight status (protein*weight status)), physical activity (bout volume (MET-mins), other daily MVPA (MET-mins)), dietary (daily carbohydrate intake), and timing (hours until midnight) variables. Interaction models for sex and category of protein intake were additional adjusted for tanner stage.

4.4. DISCUSSION

This study evaluated a unique intersection between nutrient timing and diabetes care by assessing the effects of post-exercise and daily protein intakes on post-exercise glycemia among adolescents with T1D. It was hypothesized that increased post-exercise and daily protein intake would be associated with improved TIR and reduced TBR following cessation of MVPA bouts until the following morning. No significant associations were observed between post-exercise protein intake (g or g/kg) and any primary outcome. Further, no significant interaction effects were observed between post-exercise protein intake and exercise volume, insulin regimen, or weight status, however, a significant interaction effect was observed for post-exercise protein intake and sex which indicated that increased post-exercise protein intake may be associated with improved TIR and reduce TBR among female, but not male adolescents. Additionally, daily protein intakes ≥ 1.2g/kg/day were associated with improved TIR and reduced TAR, but not TBR following MVPA bouts, with significant interaction effects observed indicating that female adolescents, those with overweight/obesity, and those on multiple daily insulin injections may experience greater increases in TIR and greater reductions in TAR with daily protein intakes >1.2 g/kg/day.

The findings of this study did not support the hypothesis that increasing post-exercise protein intake may improve glycemia following exercise among adolescents with T1D overall, but it may be a beneficial strategy among female adolescents with T1D. The timing of MVPA bouts, however, may be important to consider as most of the reported bouts of MVPA in this study occurred in the afternoon or evening. As afternoon exercise is associated with greater post-exercise hypoglycemia risk among people with T1D[16] it would be expected that if protein had a protective effect against hypoglycemia that it would be observed following afternoon exercise,[16]

however, it is also important to consider that many adolescents may have their last meal of the day in the early evening, making the size of post-exercise protein exposure somewhat limited (median intake of 34.9 (IQR: 20.9, 52.7) grams). The few studies which have examined the effects of protein on glycemia over a period of time similar to that assessed in this study utilized larger protein doses (≥60g).[134,167] It is possible that a larger protein dose may be necessary to promote changes in glycemia overnight. The findings of this study did, support the hypothesis that elevated daily protein intakes, within sports nutrition recommend daily intake levels of 1.2-2.0g/kg/day, may improve the post-exercise glycemic response, especially among individuals utilizing multiple daily insulin injections for their diabetes care, those with overweight/obesity and among female adolescents.

While research on the effects of dietary protein intake on exercise-related glycemia among people with T1D is relatively scarce, a recent laboratory-based pilot study found that a protein bolus of 50 grams following moderate-intensity exercise caused elevated glucagon, glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) levels overnight compared to water following exercise, which collectively led to reduced glucose infusion requirements to maintain euglycemia.[141] Studies in people with type 2 diabetes (T2D) have observed similar increases in the gut hormone GLP-1 as well as pancreatic polypeptide (PYY)with high protein diets which have been shown to suppress the rate of gastric emptying and therefore the rate at which blood glucose concentrations increase following a meal.[168,169] Additionally, high protein diets have been shown to improve insulin sensitivity via reductions in intra-hepatic liver triglycerides and increases in post-meal glucagon secretion among people with T2D.[170-173] It is possible that improvements in insulin sensitivity and reductions in gastric emptying rate

associated with elevated protein intakes may contribute to the improvements in post-exercise glycemia observed in this study.

These prior research findings may also help explain why people on multiple daily insulin injections, or those with overweight or obesity, experience greater improvements in post-exercise glycemia with elevated daily protein intakes. Individuals on multiple daily insulin injections have been shown to experience greater levels of post-exercise hyperglycemia compared to their peers who use insulin pumps which may be attributed in part to greater carbohydrate consumption to avoid hypoglycemia among individuals on multiple daily insulin injections who have less acute control over insulin dosing compared to insulin pump users.[161] As such, reductions in gastric emptying rate may slow the rise in glycemia following meals containing both carbohydrate and protein which could contribute to less TAR and also more TIR. Additionally, elevated adiposity among individuals with overweight or obesity has been associated with higher levels of insulin resistance in youth.[174] In fact, among adolescents and adults with T1D, fat mass has been shown to be positively related to post-exercise blood glucose and lean mass has been shown to be inversely related to post-exercise blood glucose, indicating that body composition may play an important role in the post-exercise glycemic response.[175] Changes in insulin sensitivity may have contributed to improved post-exercise glycemia to a greater extent among this population.

Differences in body composition between male and female participants may also help to explain the differences in association we observed by sex as female participants in this study had higher estimated bodyfat percentages compared to male participants (33.5% ± 5.9% vs 20.3% ± 4.2%). While we adjusted our models for estimated body fat percentage, we may not fully account for other differences in body composition, including differences in lean mass between

male and female adolescents. Additionally, previous studies in adolescents with T1D have shown that female adolescents oxidize more fat and less carbohydrate during exercise compared to males.[176] In populations without diabetes, this difference in substrate utilization among women has been attributed in part to differing progesterone and estrogen levels during the follicular versus luteal phase of the menstrual cycle and has been shown to be yield less hepatic and muscular glycogen depletion during exercise among women.[177] While speculative, these differences in substrate utilization during exercise and the potential sparing of hepatic and muscle glycogen may influence the post-exercise glycemic response among female adolescents. Studies are needed, however, to elucidate the mechanisms by which protein affects glycemia and the influence of factors such as insulin regimen, weight status and sex on this relationship in the unique metabolic context of T1D.

4.4.1. Significance for Clinical Practice

As most exercise nutrition studies among people with T1D to date have focused predominantly on carbohydrate or insulin dosing strategies to improve exercise-related glycemia, the current study addresses an important gap in the existing evidence and can inform exercise nutrition guidelines regarding the role of protein intake on exercise-related glycemia for people living with T1D. Sports nutrition guidelines currently recommend daily protein intakes of 1.2 – 2.0g/kg/day as an effective strategy for improving recovery, athletic performance, and weight management when combined with exercise training.[116,121] These guidelines were largely based on healthy populations; however, it is likely that people with T1D may experience similar benefits following these protein intake recommendations. The findings of this study suggest higher protein intakes may also help adolescents with T1D improve the post-exercise glycemic response, especially among female adolescents and also among those who do not utilize insulin

pumps in their diabetes care and those with overweight or obesity. We did, however, observe that adolescents without overweight or obesity may experience higher TBR following a higher protein diet. As such, additional counseling and monitoring may be needed to support adolescents with T1D who may choose to follow a higher protein diet to support athletic goals.

4.4.2. Challenges & Opportunities

It is important to note that data reported in this study are observational and future work is needed to establish whether a causal relationship exists between dietary protein intake and post-exercise glycemia among individuals with T1D. Additionally, as this study relied on self-reported measures of dietary intake and physical activity, it's important to note that self-reported measures are prone to recall and social desirability biases.[153,155] Specifically, dietary intake has commonly been shown to be under-reported while MVPA is often over-reported when compared to accelerometry among adolescents which may influence the number of MVPA bouts identified in this study.[153,155] The use of the multiple pass method for dietary recalls, however, has been shown to reduce bias in self-reported dietary intake and self-reported MVPA has been shown to be more reliably measured when collected by trained interviewers, as was done in the FLEX study.[62,154,157] Also, the PDPAR instrument utilized in this study has been validated against accelerometry for relative energy expenditure of physical activity ($r=0.77$, $p<0.01$) and been shown to provide reliable identification of bouts of MVPA on a previous day ($r=0.63$, $p<0.01$).[147,156] Additionally, the lack of time-stamped insulin dosing data for these analyses limits are ability to understand the role of insulin-dosing behaviors on the observed associations. While controlling for daily carbohydrate intake may help to account for bolus insulin levels, as bolus insulin doses are based on carbohydrate intake, we cannot account for basal insulin dosing or potential insulin dosing strategies which may have been implemented to reduce the risk of

exercise-related hypoglycemia. Additionally, we did not collect data related to hormone levels or menstrual cycle among participants and therefore are unable to elucidate the role these factors may play in the differing effects of protein intake on post-exercise glycemia observed in our study. However, the availability of time-stamped dietary intake, physical activity, and continuous glucose monitoring data from the FLEX study provided a unique opportunity to assess a temporal relationship between protein intake and post-exercise glycemia among adolescents with T1D.

Literature on the role of protein intake on exercise-related glycemia for people living with T1D is scarce. The findings of this study begin to address this gap in the literature and may encourage future studies to continue to explore intersections between sports nutrition and diabetes care. While promoting safe exercise through an improved glycemic response is a priority for people living with T1D, they also chose to participate in exercise for a variety of reasons including health promotion, weight management, and athletic performance. It is important that exercise nutrition guidelines aim to support both the safety and physiologic benefits of exercise to aid people with T1D in improving their health and well-being.

4.4.3. Future Research Directions

Randomized controlled trials are needed to elucidate whether a causal relationship exists between dietary protein intake and exercise-related glycemia among people with T1D and to identify potential mechanisms of action for which protein may affect the post-exercise glycemic response. Additionally, the use of mixed methods research may provide invaluable insight into practical aspects of this nutritional strategy such as perceptions of the feasibility and potential barriers to implementing this dietary approach among adolescents and adults living with T1D. Also, as the fear of hypoglycemia is a leading barrier to regular physical activity among people

with T1D, future research should aim to further evaluate the effects of following a high protein diet on the risk of hypoglycemia among people with T1D, specifically following exercise and overnight when the risk of experiencing severe hypoglycemia is heightened.[80-82] Finally, while the benefits of elevated protein intake on the adaptive response to exercise have been well documented in healthy populations[116,121], research is needed to evaluate whether these adaptive benefits are similar among people living with T1D.

Funding: The FLEX study was funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) under award number UC4DK101132.

Institutional Review Board Statement: The Flexible Lifestyles Empowering Change study was reviewed and approved by institutional review boards at clinical sites in Colorado and Ohio as well as at the coordinating site, the University of North Carolina at Chapel Hill.

Informed Consent Statement: Written informed consent was provided by the parents of all adolescents who participated in the Flexible Lifestyles Empowering Change study as well as written informed assent from all participating adolescents.

Data Availability Statement: The data in this study are open available in the NIDDK Data Repository at DOI: 10.58020/235v-4k70

Acknowledgments: We are immensely grateful too all the adolescents and their families who generously contributed their time and effort to make this research possible. We would also like to thank all the students, staff, and faculty who contributed their time and expertise to make the FLEX study possible.

Conflicts of Interest: The authors declare no conflict of interest.

CHAPTER 5. POST-EXERCISE PROTEIN INTAKE IS ASSOCIATED WITH REDUCED TIME IN HYPOGLYCEMIA FOLLOWING MODERATE-INTENSITY CONTINUOUS EXERCISE AMONG ADULTS WITH TYPE 1 DIABETES

5.1. INTRODUCTION

For those living with T1D, the risk of developing cardiovascular disease is estimated to be ten times greater than those without diabetes and is the leading cause of morbidity and mortality.[2] Intensive insulin therapy has been shown to reduce the risk of cardiovascular disease events and mortality among people with T1D by 57% and 42%, respectively, however, it has also been shown to promote weight gain.[158]Among those who gain excessive weight, the benefits of intensive insulin therapy are substantially reduced.[65] Regular exercise is central to both diabetes and weight management and promotes reductions in body mass and BMI and improvements in glycemia and cardiorespiratory fitness; however, as many as 60% of adults with T1D report not participating in any regular weekly exercise.[4] For people living with T1D, exercise is associated with an increased risk of hypoglycemia during and at least 24 hours following exercise and the fear of hypoglycemia is commonly cited as a leading barrier to participating in regular physical activity.[5,7]

Nutrition plays an important role in managing glycemia and expert consensus on carbohydrate strategies to promote improved glycemia during and following exercise have been published [19]. However, much less is known about the role of protein intake on the management of exercise-related glycemia. For the general population, the American Dietetic Association

recommends consuming 0.25-0.3g/kg of dietary protein every 3-4 hours following exercise to support recovery from and adaption to regular exercise training.[116] Among healthy adults, increasing dietary protein intake promotes numerous physiological benefits when paired with regular exercise including improved recovery times, reduced muscular soreness, improvements in muscular strength and hypertrophy and, when paired with a hypocaloric diet, greater reductions in fat mass and greater retention of lean mass.[119,120,123,125,178,179] Among people living with T1D, protein has also been shown to cause a dose-dependent elevation in blood glucose levels, peaking around 3 hours after consumption and remaining elevated for at least 5 hours and possibly as long as 12 hours.[23,24,133,134,160,167] In theory, increasing dietary protein intake following exercise among people with T1D may mitigate declining glycemia following exercise, thereby reducing the risk of hypoglycemia.

Only two studies to the authors' knowledge have examined the effects of peri-exercise protein intake on exercise-related glycemia among people with T1D.[26] A small (n=10) randomized trial of adolescents with T1D measured glycemia during moderate-intensity continuous cycling exercise performed following different nutritional strategies found that a protein supplemented breakfast consumed 2 hours prior to exercise was effective at preventing hypoglycemia during exercise compared to a standard breakfast.[26] Similarly, in a laboratory-based pilot study of 6 participants with T1D with a mean age 20.2 ± 3.1 years and average BMI of 26.7 ± 5.0, a 50g protein bolus provided 3.25 hours after 45 minutes of moderate-intensity exercise significantly reduce the glucose requirements required to maintain euglycemia overnight compared to water, indicating that post-exercise protein intake may reduce the risk of late-onset post-exercise hypoglyecmia.[141] These studies, however, were conducted in well-controlled

environments and may not fully represent the effects of protein intake in a free-living environment.

The primary aim of this study was to conduct secondary analyses of data from an acute exercise pilot study among adults with T1D to examine the relationship between post-exercise protein intake and glycemia following isoenergetic bouts of high intensity interval training (HIIT) or moderate-intensity continuous training (MICT). We hypothesized that elevating protein intake would be associated with increased time in recommended glucose range (TIR, 70-180mg/dl) and reduced time below range (TBR, <70mg/dL).

2. METHODS & MEASURES

5.2.1. Study Design

Parent Study

Secondary analyses were performed using data from the Metabolic, Hormonal, and Physiological Characterization of Isoenergetic High Intensity Interval Training and Moderate Intensity Continuous Training in Adults with Type 1 Diabetes (HI1T) study (NCT04664205, PI Smith-Ryan). Review and approval of the HI1T study was provided by the institutional review board at the University of North Carolina at Chapel Hill (IRB Number: 20-3100). Written informed consent was provided by participants prior to participating in the HI1T study. The HI1T study enrolled 14 adults (7 male, 7 female) with T1D between the ages of 18 – 51 years old to participate in a randomized controlled exercise pilot study which aimed to characterize the metabolic, hormonal and glycemic response to exercise among adults with T1D and to explore the role of physiological variables (biological sex, lean body mass, visceral fat mass) in modulating the observed responses.

Participants reported to the Applied Physiology Lab (APL) at the University of North Carolina at Chapel Hill for familiarization with study protocols and baseline testing, which consisted of body composition testing and a graded VO_2 peak test. Following their baseline visit, participants reported to the APL for three additional measurement visits consisting of supervised HIIT, MICT, or a control visit (no exercise), in a randomized order and at least 1 week apart. Exercise sessions were performed on a cycle ergometer following an overnight fast. HIIT sessions consisted of 10 one-minute intervals at 90% VO_2 peak followed by 1 minute of active recovery. MICT sessions consisted of consistent work at 65% of VO_2 peak for a time that allowed for equal energy expenditure as the HIIT sessions (typically 15-20 minutes). Blood draws were taken on arrival to the APL, immediately post-exercise and 1-hour post-exercise or just on arrival and 1 hour later for control sessions which were then sent to the Duke Molecular and Physiology Institute Metabolomics Core for analysis of metabolic metabolites and metabolic hormone levels. Additionally, participants were asked to wear a continuous glucose monitor (CGM) and wearable physical activity tracker throughout the course of the study and also provide food records for the day before, day of, and day after each visit.

5.2.2. Participants

Participants were recruited by phone or email from the UNC Endocrinology and Diabetes Clinic and UNC Student Health Center using data from the Carolina Data Warehouse to identify individuals with a type 1 diabetes diagnosis. Individuals with T1D between the ages of 18-51 years old with recent hemoglobin a1c (HbA1c) of <9%, a BMI<30 kg/m^2, a duration of diabetes of at least one year, and were otherwise healthy were eligible to participate in the HI1T study. Individuals with a physician diagnosis of active diabetic retinopathy, peripheral neuropathy with insensate feet, autonomic neuropathy, or a cardiovascular condition that would affect exercise

tolerance as well as those who were taking medications including beta-blockers, agents that affect hepatic glucose production, xanthine derivatives or any hypoglycemic agent other than insulin were excluded from participating in the HI1T study. Additionally, individuals who had experienced severe hypoglycemia, defined as requiring a third party or hospitalization, or diabetic ketoacidosis within the last 6 months, individuals who were pregnant, had severely impaired hearing or speech, were currently doing HIIT or those who used a closed-loop pump and were not willing to use manual mode were also excluded from participating in the HI1T study.

5.2.3. Measures

Demographics and Health History

Demographics and health history questionnaires were completed at participants' baseline visit from which participants self-reported their age, sex, race/ethnicity, insurance, income, education, diabetes duration, and insulin regimen.

Continuous Glucose Monitoring (CGM)

Participants were asked to either wear a study-provided intermittently scanned CGM systems (Freestyle Libre Pro, Abbot Diabetes Care Inc., CA) for the duration of the study or share CGM data from their personal CGM devices for the time period in which they participated in the HI1T study. Percent time below range (TBR, <70mg/dL), percent time in recommended glucose range (TIR, 70-180mg/dL), and percent time above range (TAR, >180mg/dL) were calculated from the cessation of exercise until 6:30 AM the following morning using raw CGM data exports following consensus guidelines.[27] As the effects of protein intake on glycemia have

been shown to last at least 5 hours following a meal[23,24,133], we chose to restrict observations to those with at least 5 hours of CGM data following exercise.

Dietary Intake Measures

Participants were asked to complete three detailed food records of everything they ate and drank on the day before, the day of, and the day after each measurement visit. Food records were completed by participants using the ASA24 2020 automated food record system.[180] Email reminders were sent to participants on each day a food record was collected. From these records, total energy intake (kcals), as well as grams of protein, carbohydrate, and fat consumed from exercise cessation until midnight on the day of study visits was quantified. As participants were asked to report to study visits following an overnight fast, participants had no dietary intake prior to or during exercise.

Physical Activity Measures

Participants were asked to wear a wearable activity tracker (Garmin vivosmart® 4, Garmin Ltd., Kansas, USA) throughout the duration of the study which provided measures such as heart rate, step count, and minutes of moderate-to-vigorous physical activity (MVPA).

Anthropometrics and Body Composition

At their baseline visit, participant's height and weight were measured using a wall-mounted stadiometer and calibrated electric scale. Body composition was also measured using a validated 4-compartment model utilizing dual energy x-ray absorptiometry[181] (GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics, Madison, WI, USA) and bioelectrical impedance analysis (InBody570, BioSpace, Seoul, South Korea) which measured overall body

fat percentage, body fat mass (kg), lean mass (kg), visceral fat mass (kg), bone mineral content (kg) and total body water.

5.2.4. Statistical Analysis

Model Selection

All statistical analyses were performed using SAS 9.4 (Cary, NC). To account for repeated measures, mixed effects regression models were used to evaluate the proposed aims using the PROC MIXED command. Due to the small sample size for these analyses, only variables which were correlated with TAR, TIR, or TBR as well as protein intake in grams or grams/kg at a p-value <0.10 were considered for inclusion as potential covariates in our models. From these potential covariates, variables that caused a ≥10% change in the point estimate or standard error of associations were included in our final models.

Primary Analyses – Post-exercise Protein Intake & Glycemia Following Exercise

An example timeline of exposures and outcomes relative to exercise sessions is provided in **Figure 5.1**. Post-exercise protein intake was defined continuously as absolute (grams) or relative (grams/kilogram bodyweight) protein intake from the cessation of exercise until midnight. Mixed effects regression models assessed the association between post-exercise protein intake and glycemia from the cessation of supervised bouts of exercise until 6:30 AM the following morning. Final models adjusted for diabetes duration (years), lean mass (kg), and carbohydrate intake (grams).

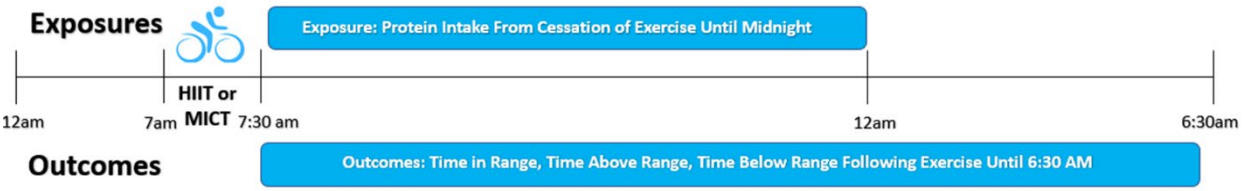


Figure 5.1. Example Timeline of Exposures & Outcomes Relative to Exercise Sessions. All exercise sessions were performed following an overnight fast.

Effect Measure Modification

Previous research has suggested that MICT and HIIT cause different post-exercise blood glucose trajectories with continuous aerobic exercise, such as MICT sessions, commonly being associated with declining glycemia, and high intensity exercise, such as HIIT or resistance exercise, commonly causing glycemia to increase during and following exercise.[182-184] It is possible that differing trajectories in blood glucose due to the type of exercise session may influence the association between protein intake and post-exercise glycemia. As such, we explored potential effect measure modification by stratifying our final models by the type of exercise session performed. Potential differences in absolute and relative protein intake as well as TAR, TIR, and TBR between HIIT & MICT sessions were assessed utilizing unadjusted mixed effects models. Due to the low sample size of this study, we also choose to graph individual responses to post-exercise on TBR by exercise modality to confirm the trends observed in primary statistical analyses and to assess for possible outlier effects.

5.3. RESULTS

Final Sample Size

Fourteen participants (7 male, 7 female) were recruited to participate in the HI1T study. As each participant reported to the Applied Physiology Lab for two exercise visits as part of the

study, we had 28 initial observations. Of these 28 observations, 5 observations from 2 participants had missing (2 observations from 1 participant) or insufficient (3 observations from 2 participants) CGM data. An additional two observations from two participants were missing dietary intake data and two observations from one participant was missing a date of diabetes diagnosis from which to determine their duration of diabetes. Our final sample size for these analyses included 19 observations from 11 participants.

Baseline Characteristics

Baseline characteristics for the participants included in these analyses is provided in **Table 1.** Participants were between the ages of 21-50 years of age with an approximately equal distribution of male and female participants and an average BMI, hemoglobin A1c (HbA1c), and diabetes duration of 25.1 ± 3.4 kg/m^2, 6.5 ± 0.8% and 17.0 ± 13.5 years. Additionally, all participants reported using a CGM at least 10 days in the past 30 days and there was a similar number of individuals utilizing continuous subcutaneous insulin infusion (54.6%) or multiple daily injects (45.4%) for their insulin regimen. Of the 11 participants included in our final analyses, 1 (9%) used a study provided Freestyle LibrePro, 10 used their personal CGM of which 6 (55%) used a Dexcom CGM, 1 (9%) used a Medtronic CGM, and 3 used a personal Freestyle LibrePro (27%).

Table 5.1. Baseline Characteristics of HI1T Participants Included in Final Analyses (n=11)

Demographic	Mean ± SD or N (%)
Age	33 ± 11.4
Female	6 (54.6)
Male	5 (45.5)
Self-Reported Race/Ethnicity	
Non-Hispanic White	8 (72.7)
Asian	2 (18.2)
Hispanic	1 (9.1)
Diabetes Care	
Diabetes Duration (years)	17.0 ± 13.5
Insulin Regimen	
Continuous Subcutaneous Insulin Infusion	6 (54.6)
Multiple Daily Injections	5 (45.4)
≥10 Days CGM Use in Past 30 Days	11 (100%)
Most Recent HbA1c (%) (n=10)	6.5 ± 0.8
Anthropometric	
Weight (kg)	73.9 ± 13.4
BMI	25.1 ± 3.4
Estimated Body Fat %	26.8 ± 7.7
Total Lean Mass (kg)	51.0 ± 10.5
Total Body Fat Mass (kg)	19.0 ± 7.5
Visceral Fat Mass (kg)	0.4 ± 0.3
Diet	
Daily Caloric Intake (kcal)	1675.2 (1417.8, 2040.6)
Percent of Daily Calories from Protein	14.9 ± 4.7
Percent of Daily Calories from Carbohydrate	40.2 ± 13.6
Percent of Daily Calories from Fat	42.5 ± 9.5
Daily Fiber Intake (grams)	11.4 (10.5, 15.6)
VO2 Peak (ml/kg/min)	31.1 ± 9.1

Continuous variables are reported as mean and standard deviation except for non-normally distributed variables, in which median and interquartile range are reported. Categorical variables are described with counts and percentages.

Results of Primary Analyses

The median intake of dietary protein following bouts of exercise until midnight was 75.0 (IQR: 57.5, 98.8) grams or 0.94 (IQR: 0.76, 1.17) grams/kilogram of bodyweight. Median TAR, TIR, and TBR following exercise sessions was 21.0% (IQR: 12.5%, 52.1%), 73.6% (IQR: 46.2%, 87.3%) and 1.3% (IQR: 0.0%, 4.1%), respectively. Results for primary analyses assessing the relationship between absolute (grams) and relative (grams/kilogram bodyweight) post-exercise protein intake on glycemia following exercise are provided in **Table 2.** While there appeared to be a trend towards lower TBR following exercise for both absolute protein intake, -1.2% (95% CI: -2.6%, 0.3%) per 20g protein (p=0.09), and relative protein intake, -1.0% (95%CI: -2.1%, 0.1%) per 0.25g/kg protein (p=0.07), these results were not statistically significant. No statistically significant associations were observed between absolute protein intake with TIR, -1.7% (95% CI: -9.3%, 5.8%) per 20g protein (p=0.60), or TAR, 2.9% (95% CI: -5.1%, 10.9%) per 20g protein (p=0.41). Similarly, no association was observed between relative protein intake and TIR, -1.5% (95% CI: -7.4%, 4.4%) per 0.25g/kg protein (p=0.55), or TAR, 2.3% (95% CI: -4.0%, 8.6%) per 0.25g/kg protein (p=0.41).

Table 5.2. Results of Mixed Effects Regression Models Assessing the Association Between Post-Exercise Protein Intake and Glycemia Following Supervised Exercise (n=11, obs=19)

	Post-Exercise Protein (grams)*			**Post-Exercise Protein (g/kg)†**		
	Estimate	**p-value**	**95% CI**	**Estimate**	**p-value**	**95% CI**
			Unadjusted Models			
Percent Time Above Range	2.7%	0.48	(-5.8%, 11.2%)	0.8%	0.76	(-5.2%, 6.9%)
Percent Time In Range	-1.7%	0.66	(-10.1%, 6.7%)	-0.1%	0.96	(-6.1%, 5.8)
Percent Time Below Range	-0.8%	0.10	(-1.9%, 0.2%)	-0.6%	0.10	(-1.3%, 0.1%)
			Fully Adjusted Models‡			
Percent Time Above Range	2.9%	0.41	(-5.1%, 10.9%)	1.8%	0.41	(-4.0%, 8.6%)
Percent Time In Range	-1.7%	0.60	(-9.3%, 5.8%)	-1.2%	0.55	(-7.4%, 4.4%)
Percent Time Below Range	-1.2%	0.09	(-2.6%, 0.3%)	-1.0%	0.07	(-2.1%, 0.1%)

*Associations are reported per a 20g dose of protein
†Associations are reported per a 0.25g/kg dose of protein
‡Models are adjusted for diabetes duration, lean mass (kg), & carbohydrate intake.

Results of Effect Measure Modification

No significant differences were observed between HIIT & MICT sessions for any of the exposures or outcomes variables (p-values >0.36). The results of stratified models are provided in **Table 3.** In stratifying final models by exercise session type, it was observed that absolute protein intake (grams) was borderline associated with reduced TBR following bouts of MICT, -1.9% (95% CI: -3.9%, 0.0%) per 20g protein (p=0.05), but not following HIIT sessions, 1.2% (95% CI: -2.4%, 4.9%) per 20g protein (p=0.42). Relative protein intake (g/kg) was not associated with TBR following MICT, -1.2% (95% CI: -3.0%, 0.6%) per 0.25g/kg (p=0.14), or HIIT sessions, 2.4% (95% CI: -1.0%, 5.8%) per 0.25g/kg (p=0.13). No significant associations were observed between absolute protein intake and TIR or TAR for MICT, -3.0% (95%CI: -21.2%, 15.2%) TIR and 4.9% (95% CI: -13.5% 23.4%) TAR per 20g protein, or HIIT, 5.3% (95% CI: -8.0%, 18.7%)TIR and -6.6% (95% CI: -18.3%, 5.25%) TAR per 20g protein. Similarly, no statistically significant associations were observed between relative protein intake and TIR or TAR for MICT , -1.1% (95%CI: -14.8%, 12.7%) TIR and 2.3% (95% CI: -12.0%, 16.5%) TAR per 0.25g/kg protein, or HIIT, -0.9% (95%CI: -17.3%, 15.5%) TIR and 2.3% (95% CI: -17.0%, 14.0%) TAR per 0.25g/kg protein. Individual Responses to Post-Exercise Protein Intake on Post-Exercise TAR, TIR and TBR are illustrated in **Supplementary Figures 5.1-5.12.**

Table 5.3. Results of Mixed Effects Regression Models Assessing the Association Between Post-Exercise Protein Intake and Glycemia Following Supervised Exercise (n=11, obs=19)

	Post-Exercise Protein (grams)*			Post-Exercise Protein (g/kg)†		
	Estimate	**p-value**	**95% CI**	**Estimate**	**p-value**	**95% CI**
Moderate Intensity Continuous Training (MICT)						
Unadjusted Models (n=10, obs=10)						
Time Above Range	-1.30%	0.85	(-17.1%, 14.4%)	-3.10%	0.53	(-14.1%, 7.8%)
Time In Range	3.00%	0.66	(-12.4%, 18.4%)	4.10%	0.39	(-6.4%, 14.6%)
Time Below Range	**-1.70%**	**0.04**	**(-3.3%, -0.1%)**	-1.00%	0.11	(-2.2%, 0.3%)
Fully Adjusted Models‡ (n=9, obs=9)						
Time Above Range	4.90%	0.5	(-13.5%, 23.4%)	2.30%	0.46	(-12.0%, 16.5%)
Time In Range	-3.00%	0.67	(-21.2%, 15.2%)	-1.10%	0.84	(-14.8%, 12.7%)
Time Below Range	**-1.90%**	**0.05**	**(-3.9%, 0.0)**	-1.20%	0.14	(-3.0%, 0.6%)
High Intensity Interval Training (HIIT)						
Unadjusted Models (n=11, obs=11)						
Time Above Range	5.00%	0.27	(-4.62%, 14.6%)	6.20%	0.21	(-4.3%, 16.8%)
Time In Range	-4.50%	0.29	(-13.8%, 4.7%)	-6.40%	0.18	(-16.3%, 3.5%)
Time Below Range	-0.50%	0.53	(-2.0%, 1.1%)	0.10%	0.86	(-1.7%, 1.9%)
Fully Adjusted Models‡ (n=10, obs=10)						
Time Above Range	-6.60%	0.21	(-18.3%, 5.2%)	-1.50%	0.82	(-17.0%, 14.0%)
Time In Range	5.30%	0.35	(-8.0%, 18.7%)	-0.90%	0.89	(-17.3%, 15.5%)
Time Below Range	1.20%	0.42	(-2.4%, 4.9%)	2.40%	0.13	(-1.0%, 5.8%)

*Associations are reported per a 20g dose of protein
†Associations are reported per a 0.25g/kg dose of protein
‡Models are adjusted for diabetes duration, lean mass (kg), & carbohydrate intake.

5.4. DISCUSSION

This study evaluated the relationship between free-living post-exercise protein intake and glycemia following isoenergetic bouts of MICT or HIIT among 11 adults with T1D. We hypothesized that higher post-exercise protein intake would be associated with improved TIR and reduced TBR following exercise sessions until the following morning. Overall, no significant associations were observed between post-exercise protein intake and post-exercise TIR, TBR or TAR. However, there was a trend towards reduced TBR with increasing protein intake ($p<0.1$). In stratified analyses, we observed a borderline significant association suggesting

increasing absolute protein intake (grams), but not relative protein intake (grams) may be associated with reduced TBR following MICT sessions (p=0.05) , but not HIIT sessions (p=0.42). No significant associations were observed between post-exercise protein intake and TIR or TAR for MICT or HIIT sessions in stratified analyses.

When examining individual responses to post-exercise protein intake and post-exercise TBR (**Supplementary Figures 5.9-5.12**), the pattern of responses appears to be in agreement with mixed effects regression results suggesting a trend towards reduced TBR following MICT with increasing post-exercise protein intake and does not appear to be affected by outlying observations. Another important takeaway from these results is the range of times spent in hypoglyecmia by participants. Nearly 1 in 4 observations demonstrated TBR greater than clinical guidelines which recommend the minimization of glycemic excursions with a target of $< 4\%$ TBR. Additionally, several observations had >8% TBR, or ~105 minutes TBR, highlighting the clinical significance of post-exercise glycemia in this population.[185]

While there didn't appear to be a trend in individual responses of increasing post-exercise protein intake on TIR following either HIIT or MICT **(Supplementary Figures 5.5-5.8),** there did appear to be a potential trend towards reduced TAR with increasing post-exercise protein intake following HIIT **(Supplementary Tables 5.2 & 5.4),** but not MICT **(Supplementary Tables 5.1 & 5.3),** that may have been affected by an outlying observation. After rerunning stratified analyses following removal of this potential outlier, however, associations for relative (-5.8%, p=0.3) and absolute protein intakes (-6.6%, p=0.11) on TAR following HIIT remained statistically non-significant.

The findings of this study suggest that elevating protein intake following moderate-intensity continuous training, as is recommended by sports nutrition guidelines[116], may reduce

the risk of experience post-exercise hypoglycemia following aerobic exercise sessions until the following morning. A 20g dose of exercise protein intake, approximately the amount provided by 3oz of chicken or 1 scoop of a protein supplement, was associated with a -1.9% (95% CI: -3.9%, 0.0%) reduction in TBR. Given that the median TBR following exercise sessions was 1.3% (IQR: 0.0%, 4.1%) which is equivalent to ~15.5 minutes, a reduction of 1.9% or ~24.5 minutes is clinically significant. While no association was observed between post-exercise protein intake and glycemia following bouts of HIIT, this may be explained by differences in the glycemic response during and following exercise between these different exercise modalities. While results have been inconsistent, a meta-analysis of exercise trials in adults with T1D suggested that intermittent high intensity exercise, such as HIIT, may cause less of a decline in blood glucose levels following exercise compared to continuous moderate intensity exercise, possibly due to an increased counter-regulatory response to higher intensity activity.[183] Additionally, the prandial state in which HIIT exercise appears to modulate the glycemic effect observed following exercise among people with T1D.[186-188] Recent studies have observed that when HIIT is performed in a fasted state, as in this study, blood glucose levels tend to increase following exercise whereas they tend to decrease following HIIT exercise performed in a post-prandial state.[186-188]

While research investigating the effects of post-exercise protein intake on glycemia among individuals with T1D is scarce, a study by Paramalingam et al found that a 50g bolus of protein following moderate-intensity continuous exercise reduced the glucose requirement to maintain euglycemia overnight compared to water.[141] This effect was attributed in part to an increase in glucagon which may stimulate hepatic glucose production.[141] Another study among adolescents with T1D (n=10) observed that a protein-supplemented breakfast consumed two

hours prior to exercise raised blood glucose levels at the onset of exercise compared to a standard breakfast, leading to a reduced occurrence of hypoglycemia during exercise.[26] While speculative, it is possible that increasing hepatic glucose production, possibly through gluconeogenesis utilizing specific amino acids from high protein meals, may help to mitigate the decline in glycemia observed following exercise. More research is needed, to determine whether a causal relationship exists between post-exercise protein intake and the risk of hypoglycemia following exercise and to provide insights into potential mechanisms by which post-exercise protein intake effects the post-exercise glucose response.

Challenges and Opportunities

A primary limitation of this study is, as it uses data from a pilot study, there was a limited sample size to evaluate the aims of the study (n=11, obs=19). In addition to limiting the statistical power of our analyses, the small sample size restricted the number of potential covariates that could be incorporated in our statistical models, thus increasing the potential for residual confounding. Additionally, while the findings of this study provide insights which may inform future studies, the observational nature of our analyses limits our ability to evaluate a causal relationship between post-exercise protein intake and post-exercise glycemia. As individuals in this study had very good glycemic control at baseline (mean HbA1c 6.5% ± 0.8%) and reported lower levels of kcals from protein compared to other studies in adults with T1D (T1D Exchange: 18.2% ± 0.6% vs HI1T: 14.9% ± 4.7%), there is a potential that our results may be influenced by potential selection bias which may bias our results away from the mean.[189] Furthermore, self-reported measures of dietary intake are prone to under-reporting[190], however, the extent of mis-reporting has been shown to be reduced when the multiple pass method is utilized as in the ASA-24 system that was utilized in this study.[191] Additionally, while

carbohydrate intake was controlled for which may help to account for bolus insulin provision which is based on carbohydrate intake, the lack of insulin dosing data limits our ability to assess whether changes in insulin-dosing behaviors may have influenced the observed results. Finally, since participants in this study were allowed to either use a study provided CGM or share the data from their own personal CGM, there may be bias due to differences in the error of measurement of interstitial blood glucose by CGM type.

This study also had had several strengths. One strength of this study was the provision of supervised, iso-energetic bouts of HIIT and MICT exercise sessions which reduces the risk of confounding related to differences in energy expenditure between bouts of exercise. Additionally, the use of continuous glucose monitoring throughout the study provided the opportunity to examine glycemia over a fuller extent of the time period for which the risk of hypoglycemia is elevated following exercise. Additionally, while observational in nature, these data allowed us to address an important gap in the literature relating to the role of post-exercise protein intake on post-exercise glycemia.

Relevance for Clinical Practice

Increasing protein intake following exercise is recommended by sports nutrition guidelines for promoting numerous physiological adaptations to exercise trianing.[116] Following aerobic exercise, increasing protein intake has been associated with reduced muscle damage and soreness and improved recovery following exercise.[123,192-194] Additionally, following resistance exercise, elevating protein intake has been associated with improvements in muscular strength and hypertrophy[178,195], and when paired with a caloric deficit, high protein diets may promote reductions in fat mass and retention of lean mass during weight loss.[125,127] While these findings have been observed largely in healthy populations, it is likely people with T1D may experience

similar adaptive benefits to increasing protein intake post-exercise. Our results suggest that, among people with T1D, following this same nutritional strategy may potentially reduce the risk of post-exercise hypoglycemia, specifically following MICT exercise. Our results also suggested that increasing post-exercise protein intake isn't associated with any detrimental changes in glycemia following either MICT or HIIT sessions. While more research is needed, increasing protein intake post-exercise may pose a promising strategy for both mitigating the risk of hypoglycemia following exercise and improving the adaptive response to exercise among people living with T1D.

Future Directions

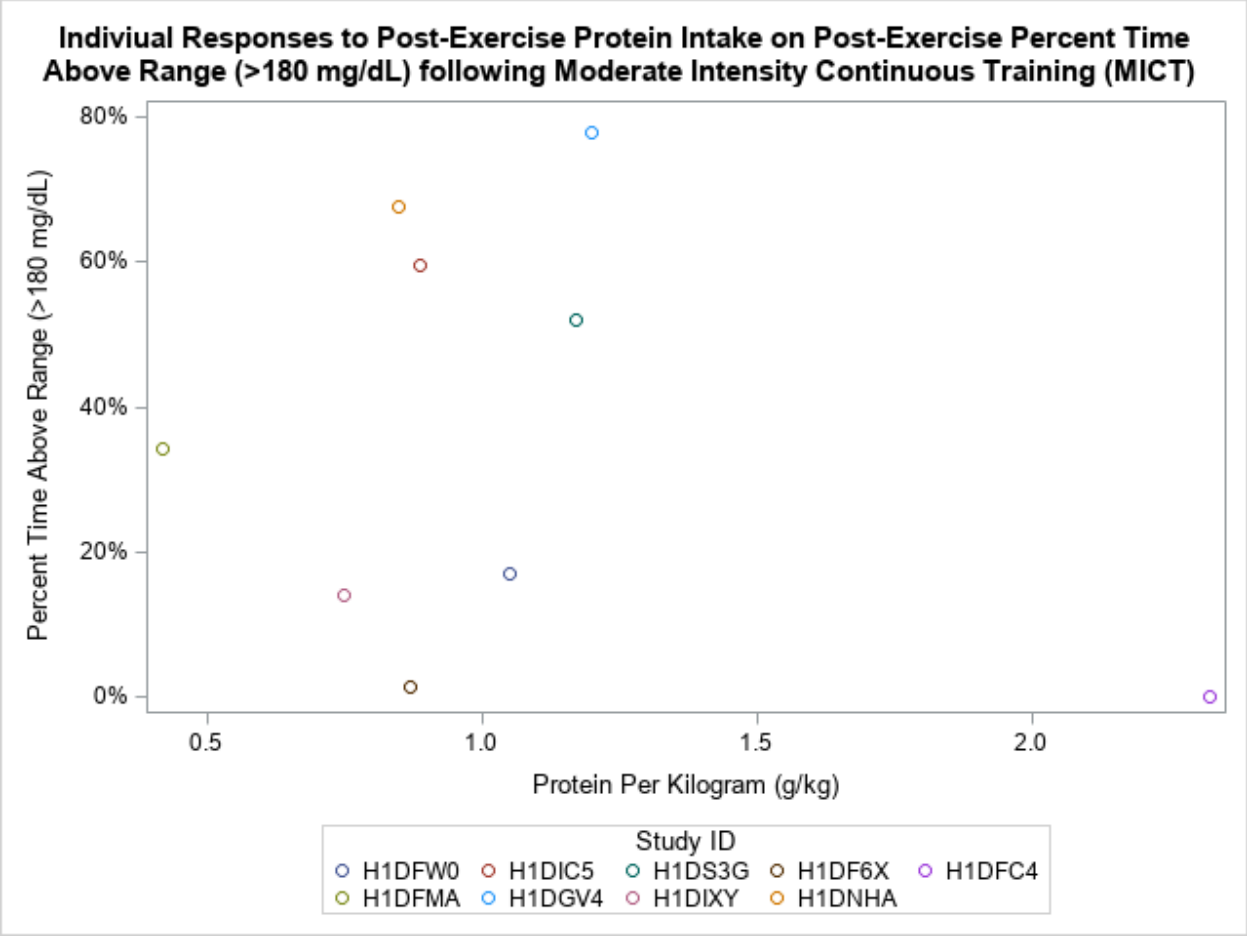
Research in the field of peri-exercise protein intake and exercise-related glycemia among people with T1D is very limited but warrants further study. Specifically, randomized controlled studies are needed to establish whether a causal relationship exists between post-exercise protein intake and post-exercise glycemia among adults with T1D. As fear of hypoglycemia is a leading barrier to regular participation in physical activity for people with T1D, identification of nutritional strategies that may help to address this fear while also providing other potential health and performance benefits would have significant implications for clinical practice. While current strategies around carbohydrate consumption are effective in treating or preventing hypoglycemia[19], young adults with T1D have reported that the need to consume carbohydrate to prevent hypoglycemia with exercise can create a feeling of futility around exercise when weight management is a primary goal for exercise.[6] Future work should strive to bridge the fields of sports nutrition and diabetes care to identify nutritional strategies that may aid adults in exercising safely while also supporting the goals that have motivated them to engage in more regular exercise.

Acknowledgements: We are very grateful to all the participants whose time, sweat and dedication to improving the lives of people with T1D made this study possible. We would also like to thank Taylor Morrison and Hannah Cabre whose hard work and expertise were invaluable to the successful completion of the HI1T study.

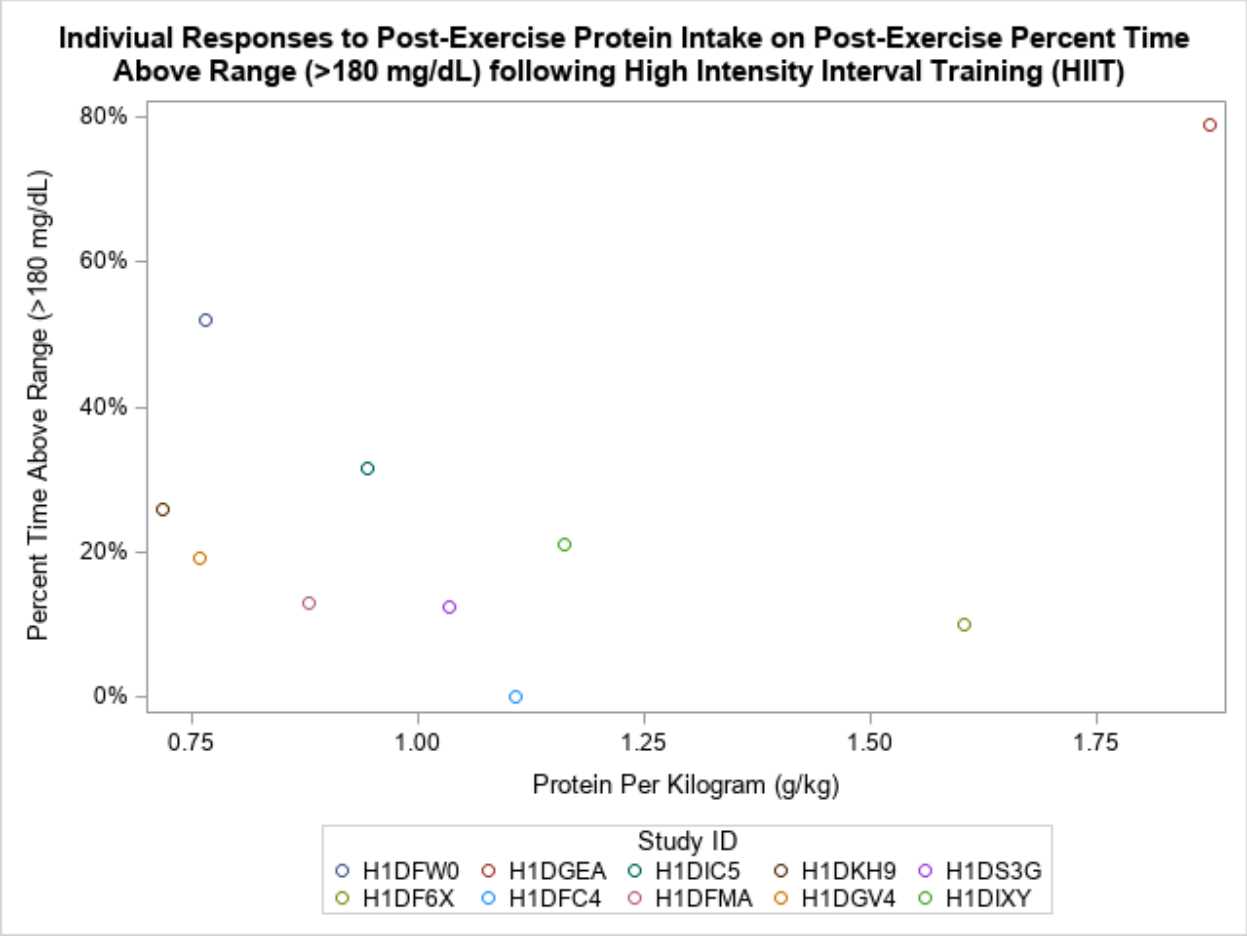
Conflicts of Interest: The authors declare no conflict of interest.

Funding: The HI1T study was funded by a pilot grant from the North Carolina Diabetes Research Center (NCDRC).

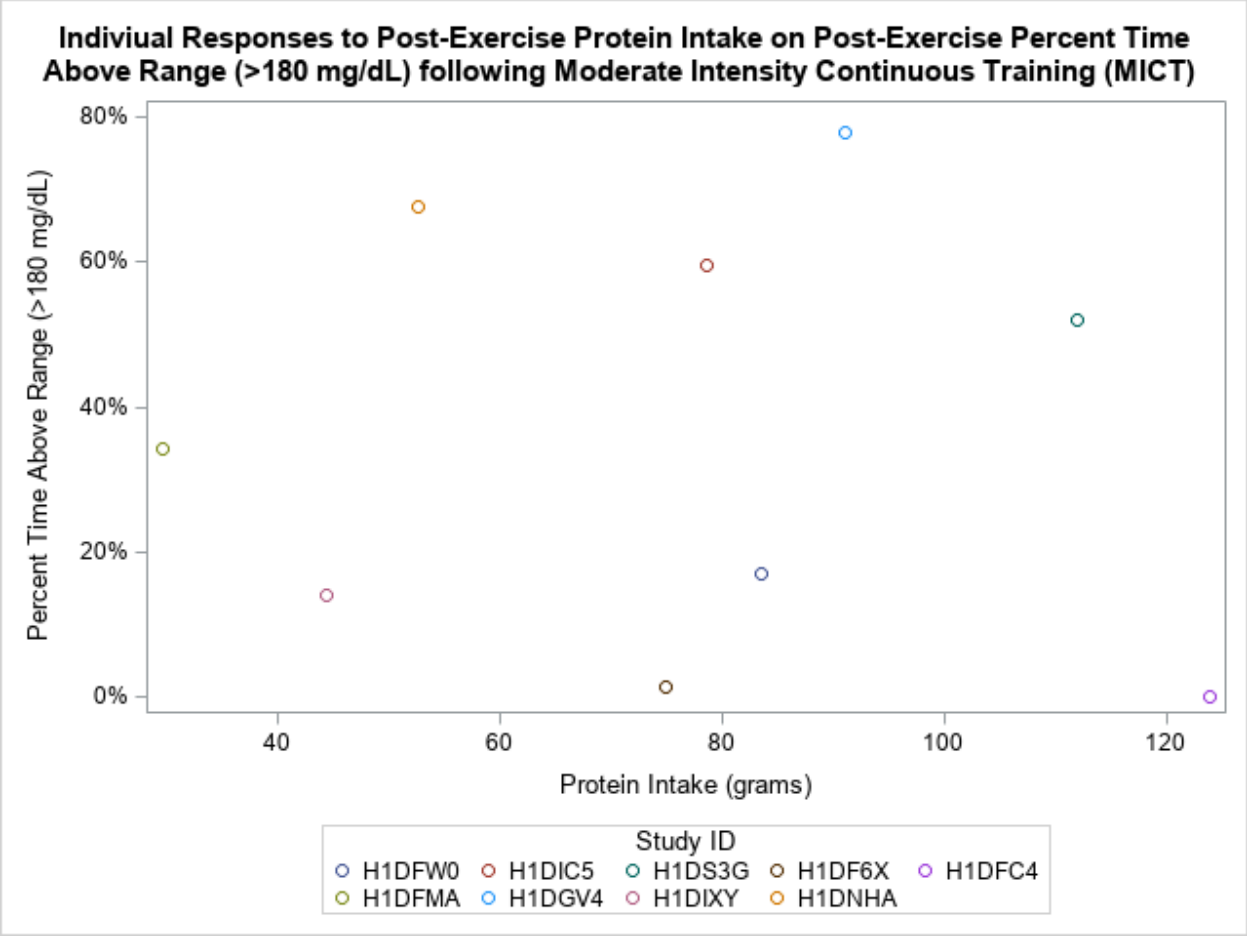
Supplementary Figures



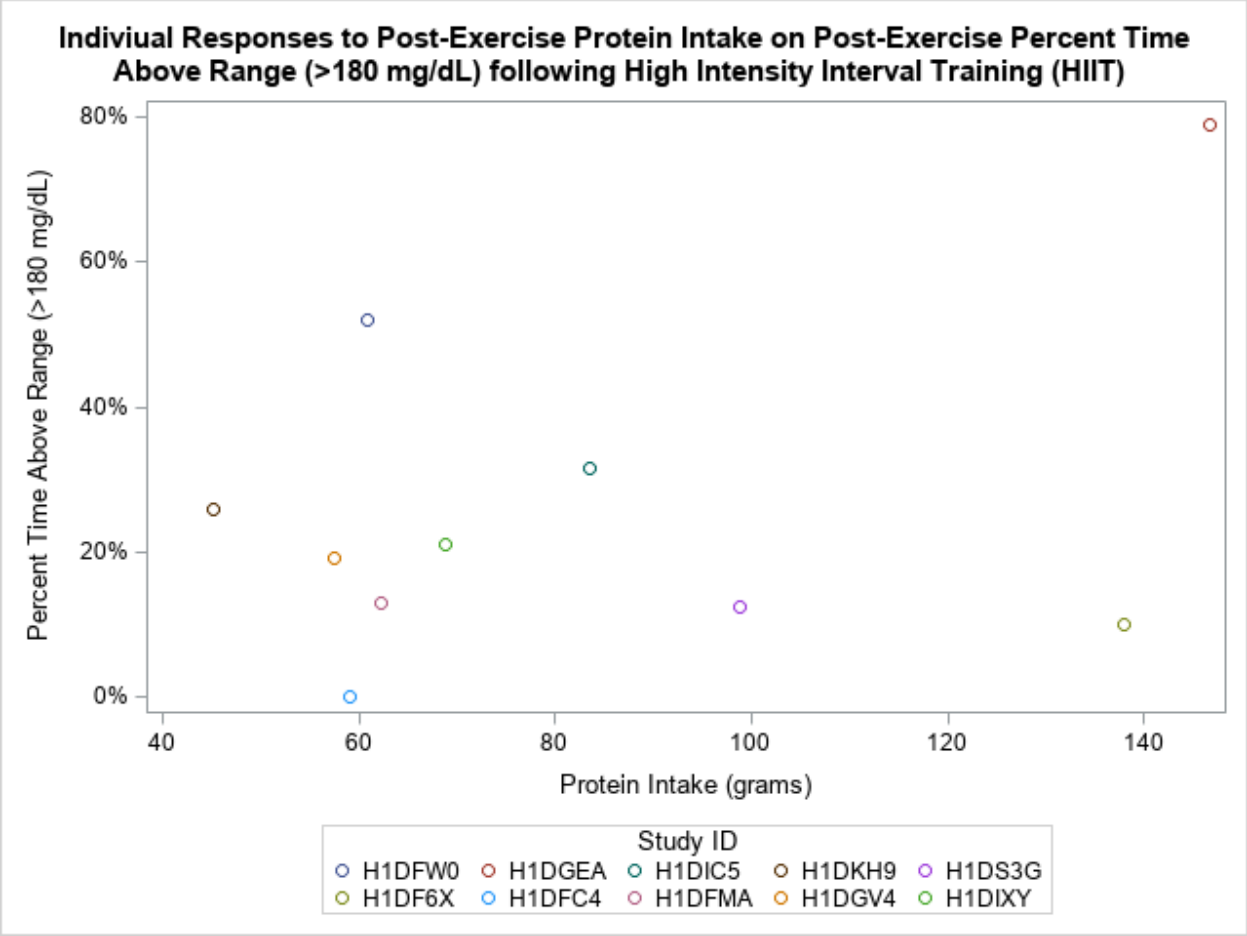
Supplementary Figure 5.1. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (g/kg) on Post-Exercise Percent Time Above Range (>180mg/dL) Following Moderate-Intensity Continuous Training (MICT).



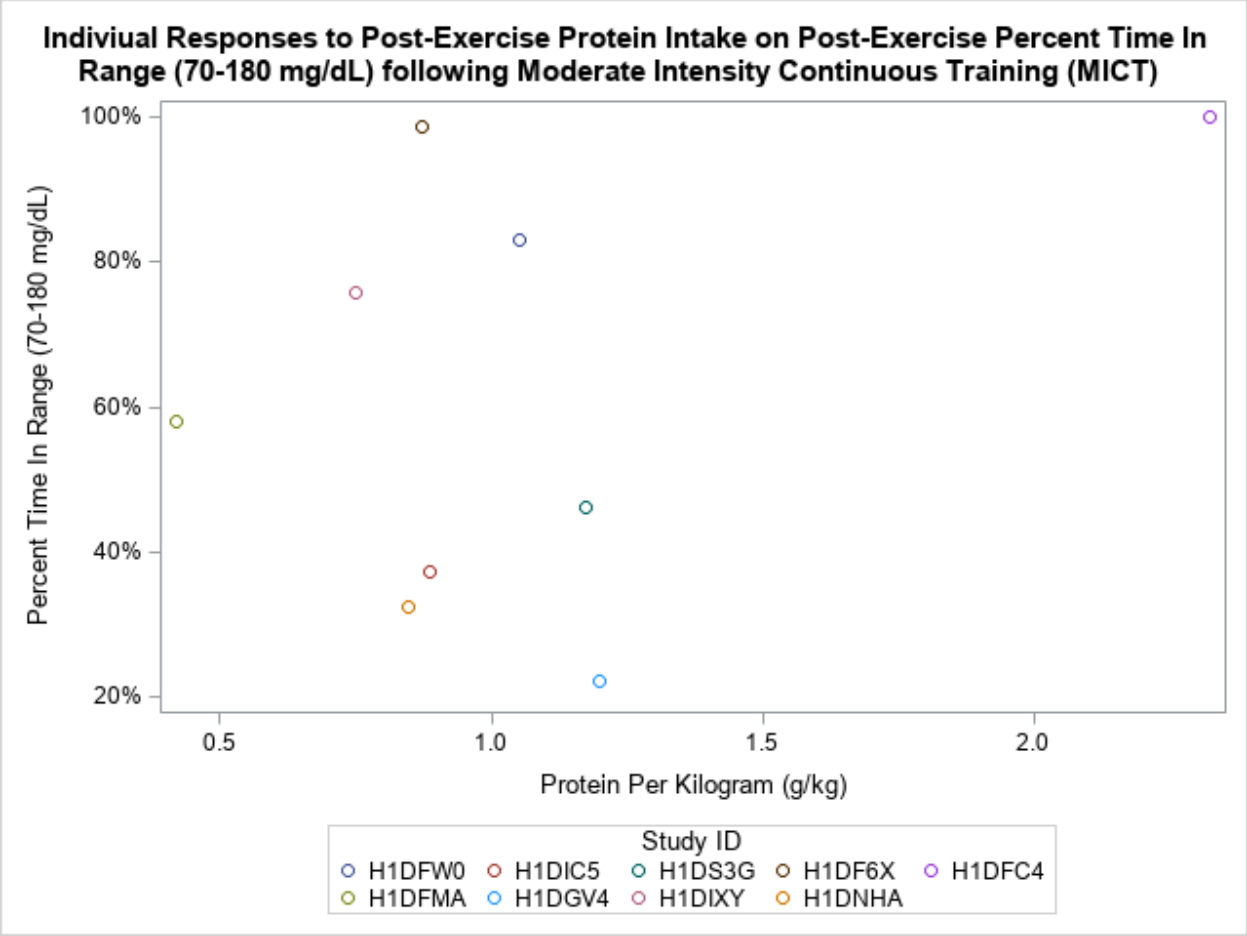
Supplementary Figure 5.2. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (g/kg) on Post-Exercise Percent Time Above Range (>180mg/dL) Following High Intensity Interval Training (HIIT).



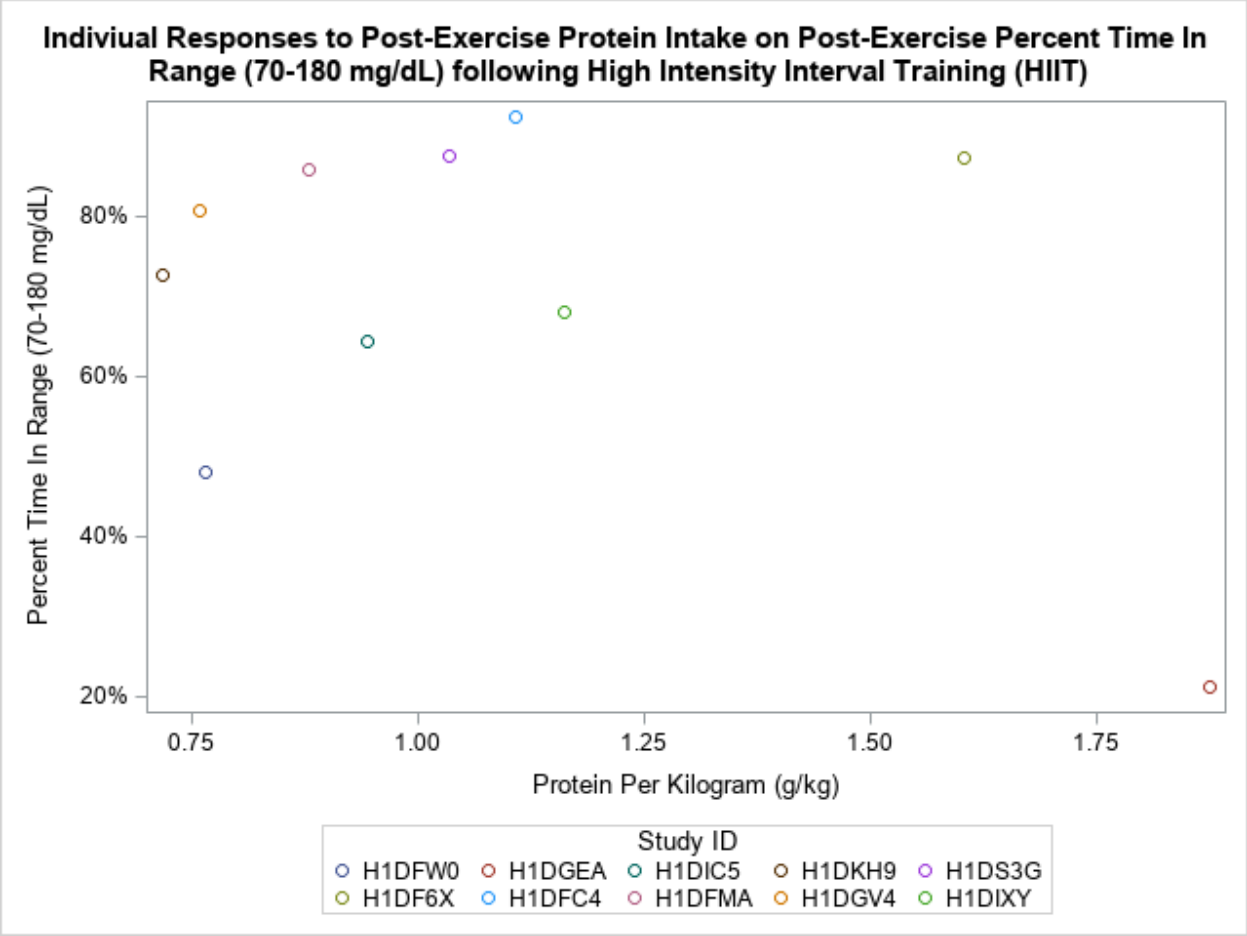
Supplementary Figure 5.3. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (grams) on Post-Exercise Percent Time Above Range (>180mg/dL) Following Moderate-Intensity Continuous Training (MICT).



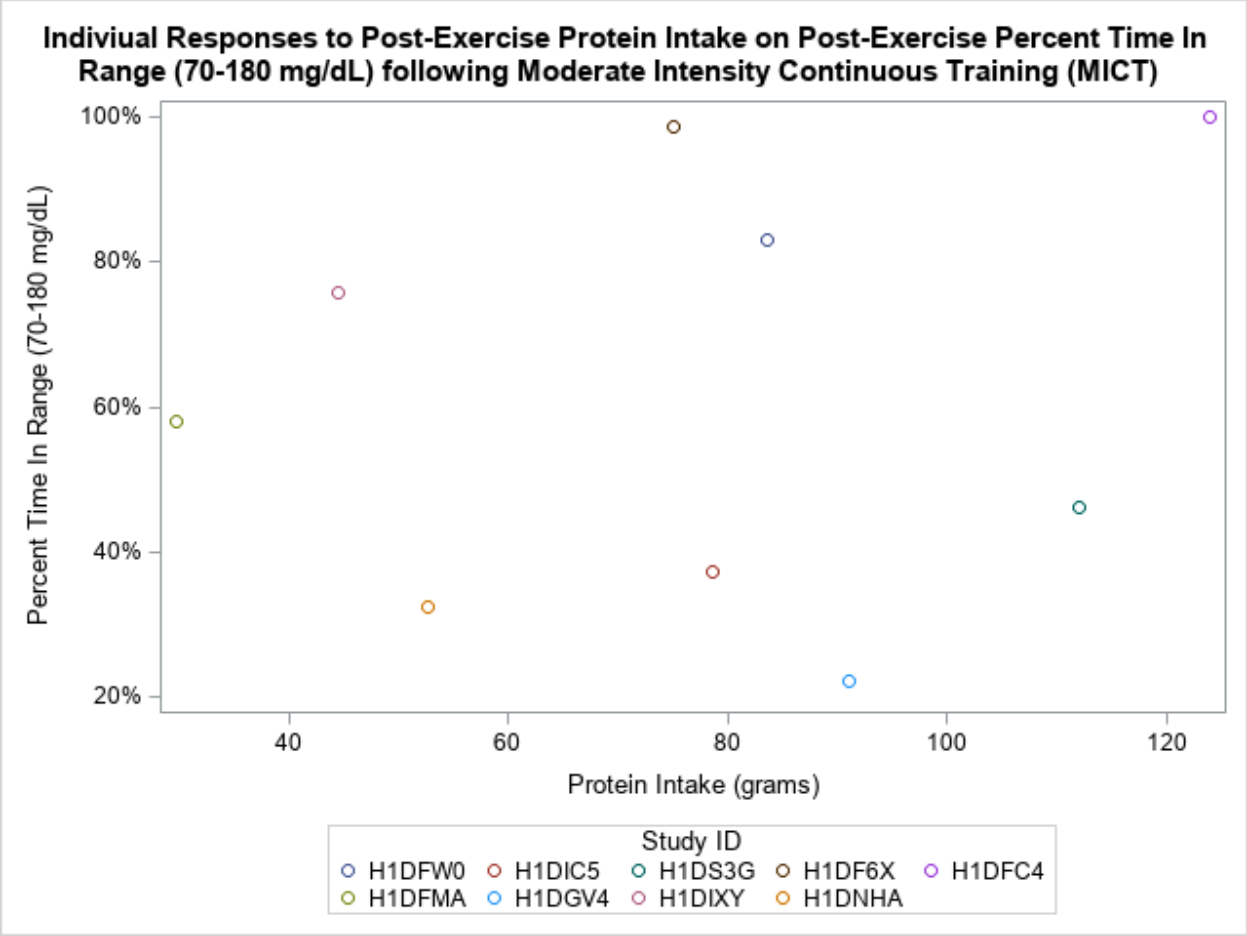
Supplementary Figure 5.4. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (grams) on Post-Exercise Percent Time Above Range (>180mg/dL) Following High Intensity Interval Training (HIIT).



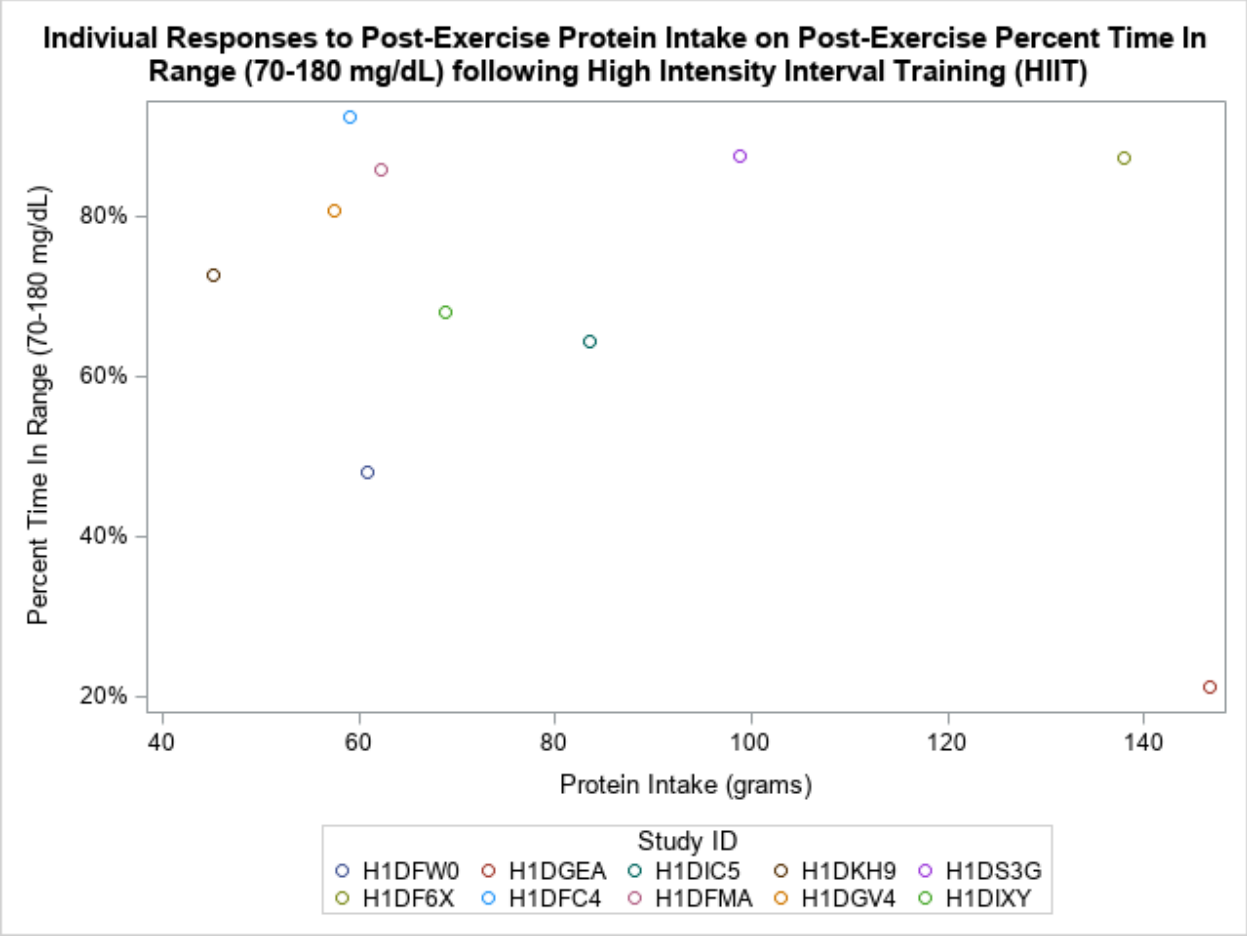
Supplementary Figure 5.5. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (g/kg) on Post-Exercise Percent Time In Range (70 - 180mg/dL) Following Moderate-Intensity Continuous Training (MICT).



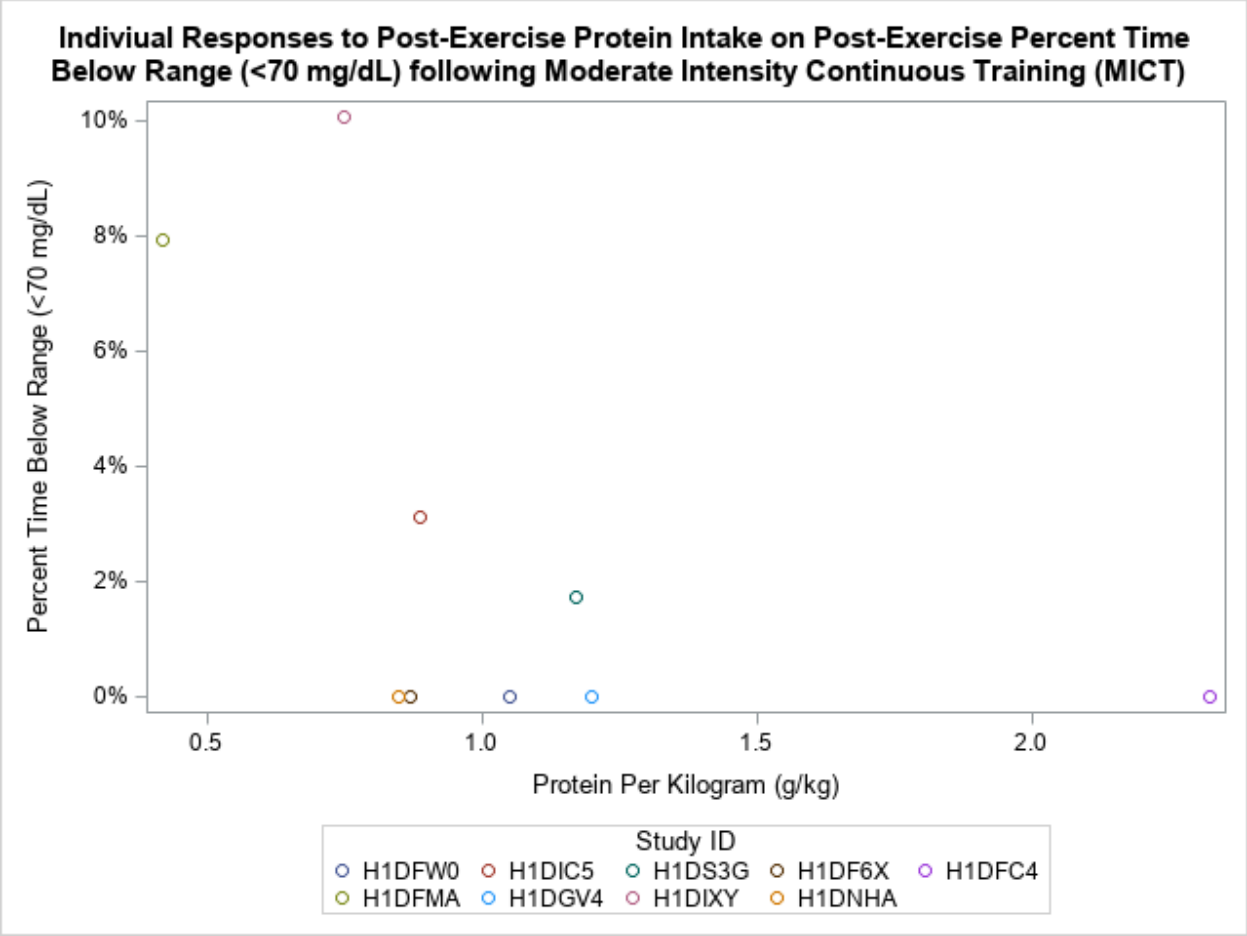
Supplementary Figure 5.6. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (g/kg) on Post-Exercise Percent Time In Range (70 - 180mg/dL) Following High Intensity Interval Training (HIIT).



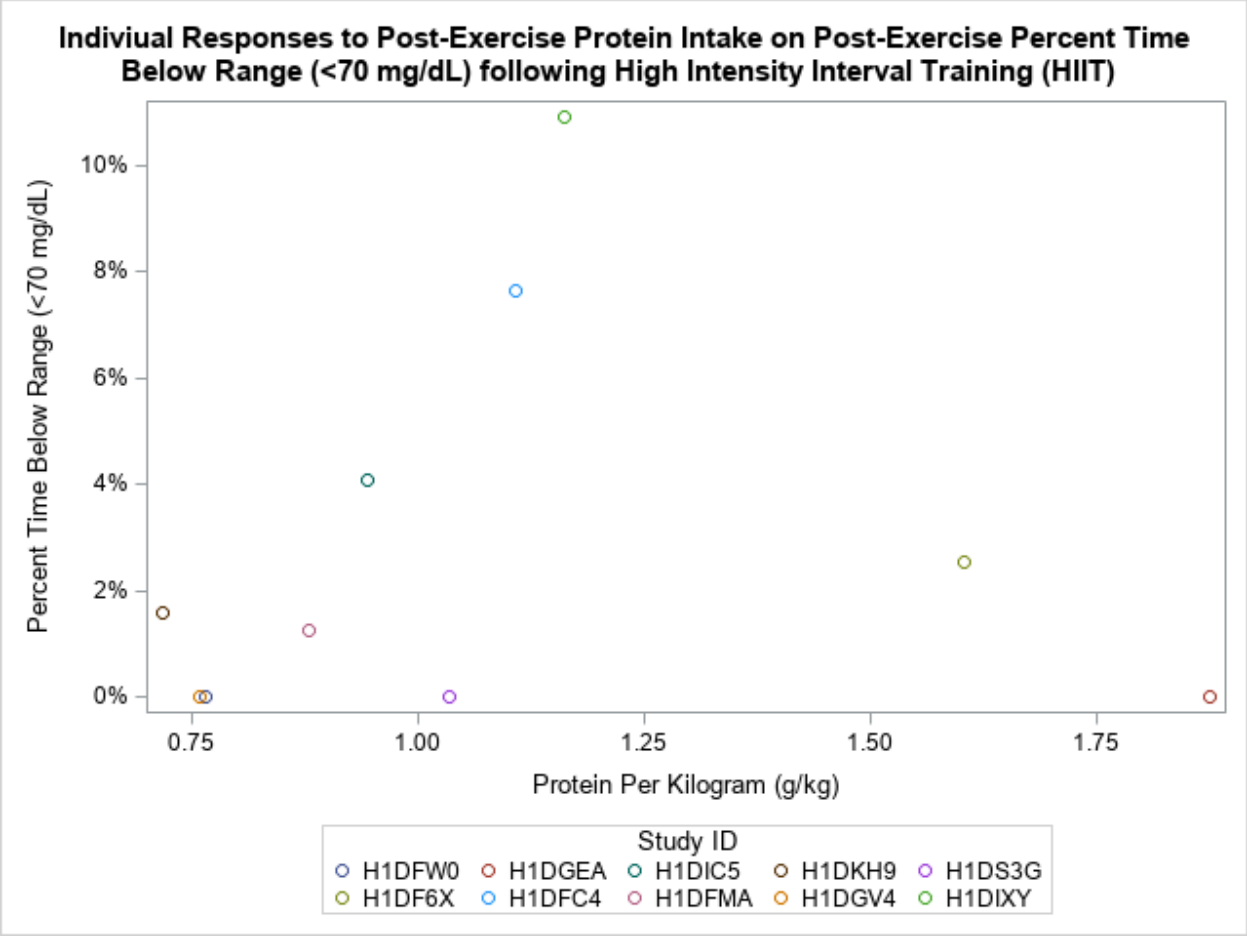
Supplementary Figure 5.7. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (grams) on Post-Exercise Percent Time In Range (70 - 180mg/dL) Following Moderate-Intensity Continuous Training (MICT).



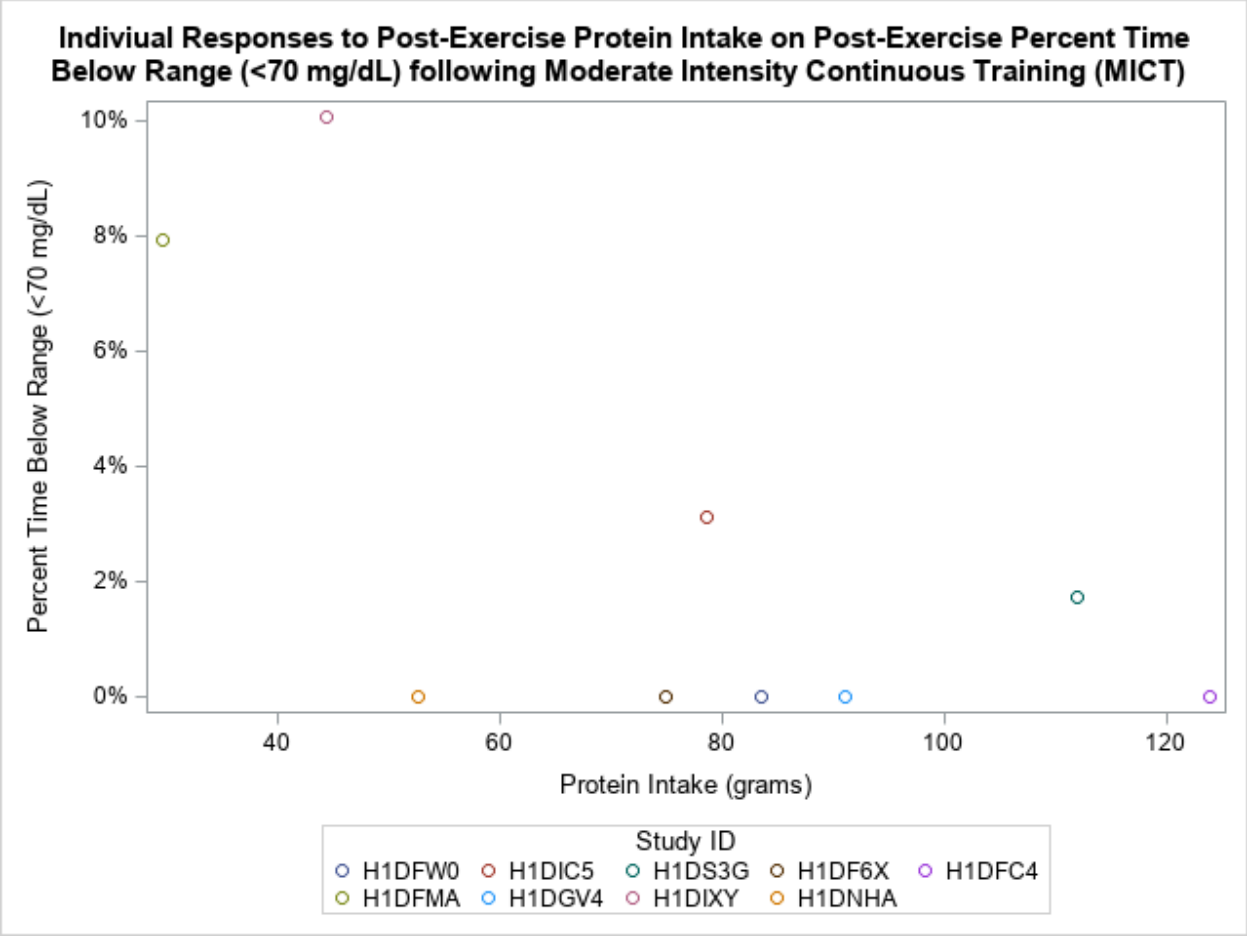
Supplementary Figure 5.8. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (grams) on Post-Exercise Percent Time In Range (70 - 180mg/dL) Following High Intensity Interval Training (HIIT).



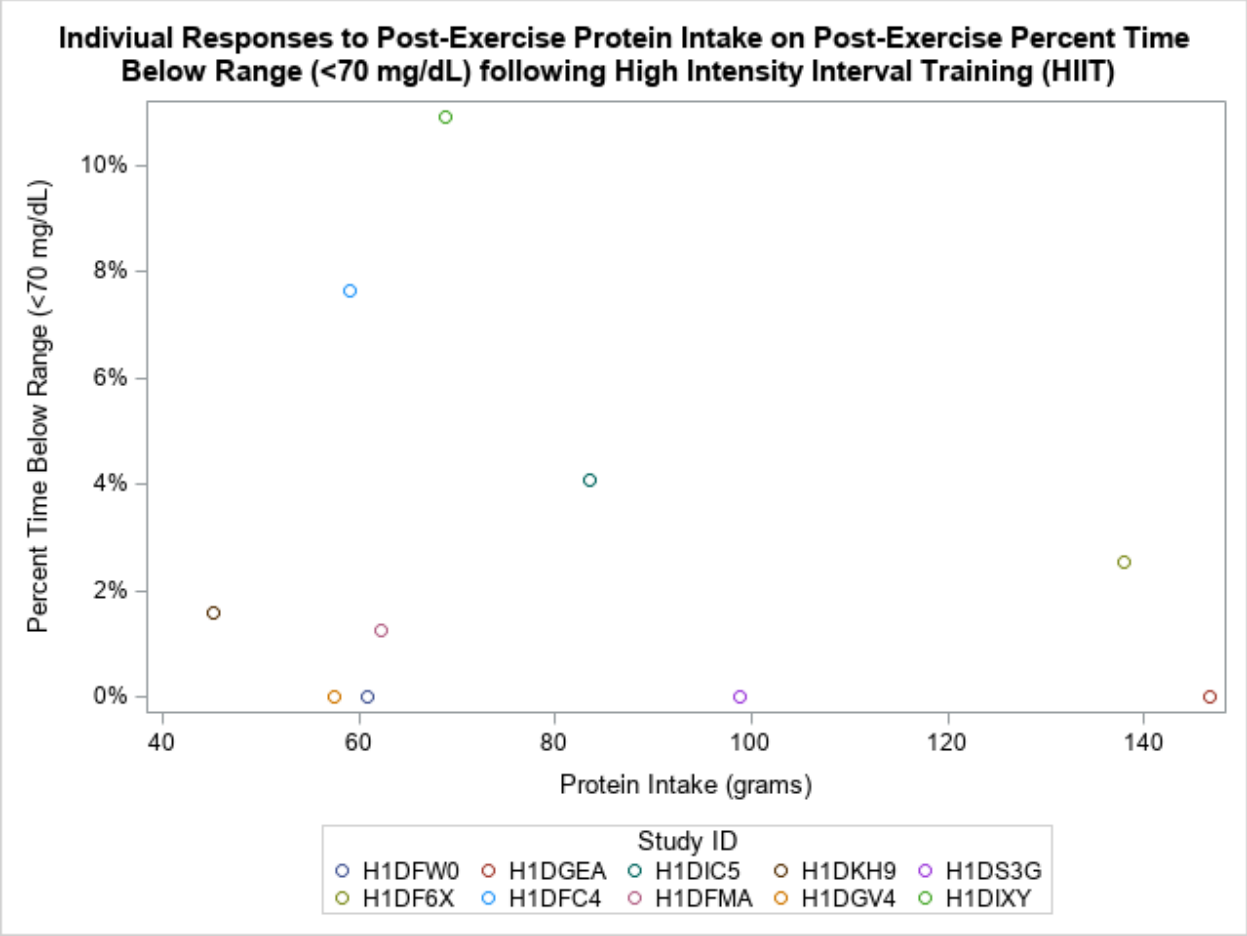
Supplementary Figure 5.9. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (g/kg) on Post-Exercise Percent Time Below Range (<70 mg/dL) Following Moderate-Intensity Continuous Training (MICT).



Supplementary Figure 5.10. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (g/kg) on Post-Exercise Percent Time Below Range (<70 mg/dL) Following High Intensity Interval Training (HIIT).



Supplementary Figure 5.11. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (grams) on Post-Exercise Percent Time Below Range (<70 mg/dL) Following Moderate-Intensity Continuous Training (MICT).



Supplementary Figure 5.12. Scatter Plot of Individual Responses to Post-Exercise Protein Intake (grams) on Post-Exercise Percent Time Below Range (<70 mg/dL) Following High Intensity Interval Training (HIIT).

CHAPTER 6. EVALUATION AND SYNTHESIS

This chapter provides an overview and synthesis of the collective findings of this dissertation work as well as its limitations, strengths, and proposed future directions.

6.1. Overview of Dissertation

Collectively, the three dissertation studies presented explored a novel intersection between the fields of diabetes care and sports nutrition, assessing the role of peri-exercise protein intake on glycemic control during and following physical activity among both adolescents and adults living with type 1 diabetes. Chapter 3 demonstrated that consuming a meal containing at least 10g or 0.125g/kg bodyweight of dietary protein within 4 hours prior to a bout of MVPA was associated with a reduced time spent below range during, but not follow MVPA bouts among adolescents with T1D. Chapter 4 followed up on these analyses by assessing the role of post-exercise and daily protein intake on post-exercise glycemia. While no association was observed between post-exercise protein intake and post-exercise glycemia overall, increasing post-exercise protein intake was associated with a reduction in TBR following MVPA bouts among female adolescents. Further, consumption of a high protein diet, within sports nutrition guidelines of 1.2-2.0g/kg/day, was associated with an overall reduction in TAR and improvement in TIR among adolescents with T1D, with the greatest effects observed among female adolescents, those with overweight or obesity, and those utilizing multiple daily injections for their insulin regimen. In Chapter 5, while limited by a small sample size, we observed a trend towards reduced TBR following supervised bouts of exercise for moderate-

intensity continuous training, but not high intensity interval training, among adults with T1D and optimal glycemic control. Together, these studies provide evidence that following sports nutrition guidelines for protein intake may also improve the glycemic response during and following exercise among individuals with type 1 diabetes.

6.2. Limitations of Dissertation

Limitations of each individual study are provided within their respective chapters (see Sections 3.4, 4.4, and 5.4). There are several limitations to this dissertation work as whole that warrant discussion.

While the data utilized in these studies provided an opportunity to explore these unique and relatively unexplored research questions, it is important to note that these analyses were exploratory and observational in nature. The associations observed in these analyses will inform future trials; however, the studies from which these data were derived from were not designed to evaluate these specific research questions and therefore do not provide sufficient evidence of a causal relationship between dietary protein intake and exercise-related glycemia. Additionally, while we know the mode of insulin delivery, we lack detailed insulin dosing data in these studies which limits our ability to understand changes in insulin dosing that may have been made in response to exercise or dietary intake which poses a risk of residual confounding bias. Additionally, these studies relied upon self-report data of dietary intake, and in Chapters 3 & 4, self-reported physical activity measures, and therefore the timing and quantity of these variables may be affected by recall or social desirability biases.[153,155]

While reports of levels of mis-reporting among people with T1D is limited, a study conducted among children and adolescents with T1D found that when compared to predicted

energy expenditure, self-reported energy expenditure was under-reported among one third of participants and over-reported among only 10% of participants.[196] Additionally, older age, male sex and higher BMI-z score were all associated with an increased risk of under-reporting.[196] To the authors knowledge, no studies have evaluated the extent of bias in reporting of protein intake, however, in populations without T1D, protein intake tends to be under-reported to a lesser extent than energy intake and 24-hour dietary recalls tend to provide stronger correlations with urinary nitrogen than food frequency questionnaires.[197] In a pooled analysis of validation studies which compared self-reported intakes of dietary protein intake to urinary nitrogen analysis, the average bias in reporting of absolute protein intake was relatively small (-5%), but had considerable variability across studies (range -21% to 20%).[197] As carbohydrate intake, and not protein, is the main focus of current nutritional approaches to glycemic management, it is likely that the mis-reporting of dietary protein intake in this study would be non-differential and therefore likely biased toward the null.[198,199]

Finally, in Chapter 5, our sample size was very limited which affected the statistical power of our analyses. Prior to beginning this dissertation work, we simulated 2 data points from each of the 14 HI1T participants assuming within-person errors correlated at r=0.4 and estimated that we had 80% power to detect a 12.4% change in TIR or TAR and a 5.9% change in TBR. As such, while our analyses were strengthened by the additional control of utilizing supervised isocaloric bouts of exercise performed after an overnight fast, it's likely that our analyses were underpowered to detect a significant association between dietary protein intake and post-exercise glycemia. Additionally, it is important to note that the participants of the HI1T study had very well-controlled glycemia (average HbA1c 6.5% ± 0.8%) which may indicate an elevated risk of

selection bias which may bias our results away from the null as lower HbA1c has been shown to be a predictor of a heightened risk of experiencing hypoglycemia.[82,200-202]

6.3. Strengths of Dissertation

A major strength of this body of work is the incorporation of data from two different studies which recruited distinct populations, allowing us to examine the effects of protein intake on exercise-related glycemia among two distinct groups of people with T1D, adolescents with suboptimal glycemic control and adults with well-controlled glycemia. While the results of Chapter 5 were likely limited by the small sample size, we were able to observe trends that may highlight how the effects of protein intake on exercise-related glycemia may differ in different contexts. Among adolescents with suboptimal glycemic management, we saw that protein intake consumed within 4 hours prior to exercise was protective against experiencing hypoglycemia during exercise, which is likely due to the mild hyperglycemic effect of protein intake among people with T1D. Interestingly, we also observed that overall daily protein intake was associated with a substantial reduction in TAR and improvement in TIR following exercise, specifically among adolescent females, those with overweight or obesity, and those utilizing MDII, suggesting that, among these populations, consuming more protein throughout the day may have a glucose lowering effect that may lead to beneficial improvements in post-exercise glycemia. It is possible that, since this population of adolescents with T1D had suboptimal glycemic control, that consuming more protein throughout the day benefitted them due to mechanisms outside of the direct effect of protein on glycemia, such as slowing of gastric emptying rate or improvements in insulin sensitivity that have been associated with a high protein diet among other populations.[168,170-172]

In contrast, among adults with T1D and well-controlled glycemia, we saw a trend towards less time in hypoglycemia following exercise, specifically following moderate-intensity continuous exercise, with greater post-exercise protein intake. While the sample size in this study limited our statistical power, it was also strengthened by the use of supervised bouts of isocaloric exercise following an overnight fast. Populations with greater glycemic control have been shown to be more likely to experience hypoglycemia[200] and moderate-intensity continuous training has also been shown to cause a decrease in blood glucose levels to a greater extent than high intensity interval training in some studies.[183] This may explain why, among this population, greater daily protein intake wasn't associated with reductions in hyperglycemia as observed in the FLEX study, but rather trended towards being protective against hypoglycemia. Collectively, these results provide invaluable initial steps in understanding the role of dietary protein intake on glycemic management with physical activity and the role that weight status, sex, insulin regimen and exercise type may play in this relationship. These data will help to inform exercise nutrition guidelines for people with T1D as well as future studies which can more rigorously test the relationship between pre-exercise, post-exercise, and daily protein intakes on exercise related glycemia among both adolescents and adults with T1D.

6.4. Proposed Future Studies

While this body of work provides a first step in understanding the role of protein intake on exercise-related glycemia for people living with T1D, more research is needed to rigorously evaluate the role of peri-exercise and daily protein intake on exercise-related glycemia among people with T1D. The following section describes three studies which may build upon the findings of this body of work and contribute valuable new data on the role of peri-exercise and

daily protein intake on glycemic, metabolic and physiological outcomes among people with T1D.

6.4.1 Evaluating the Role of Pre-Exercise Protein Intake on Glycemic, Metabolic, and Skeletal Muscle Physiology Outcomes

In Chapter 3, we identified a significant association indicating dietary protein doses of at least 0.125g/kg bodyweight consumed within 4 hours of MVPA may reduce TBR following physical activity. This is in agreement with feeding studies which have shown that, when consumed as part of a mixed meal, doses as small as 12.5g of dietary protein can induce a hyperglycemic effect among people with T1D.[23] The consumption of a meal containing at least 1g/kg of carbohydrate within 1-4 hours prior to exercise is recommended for both endurance performance exercise performance as well as hypoglycemia prevention.[19,116] While studies examining the effects of adding protein to a carbohydrate-containing meal prior to exercise are somewhat limited, a few studies have suggested that the addition of protein intake prior to exercise may also promote improved muscle recovery following exercise which may be due to the protein synthetic effects of protein intake.[122,123,203] Doses of 0.25-0.3g/kg (~20-40g) have been proposed as an optimal dose for maximizing the rate of muscle protein synthesis in health populations.[116]

Based on these findings, we would propose a randomized cross-over controlled trial to evaluate the role of carbohydrate alone or carbohydrate plus low-dose (0.125g/kg) or optimal dose (0.25g/kg) protein compared to non-caloric placebo on glycemia, metabolomic, and skeletal muscle factors during and following moderate-intensity cycling exercise among adults with T1D

and a recent HbA1c < 13%. The proposed study design is provided in **Figure 6.1.**

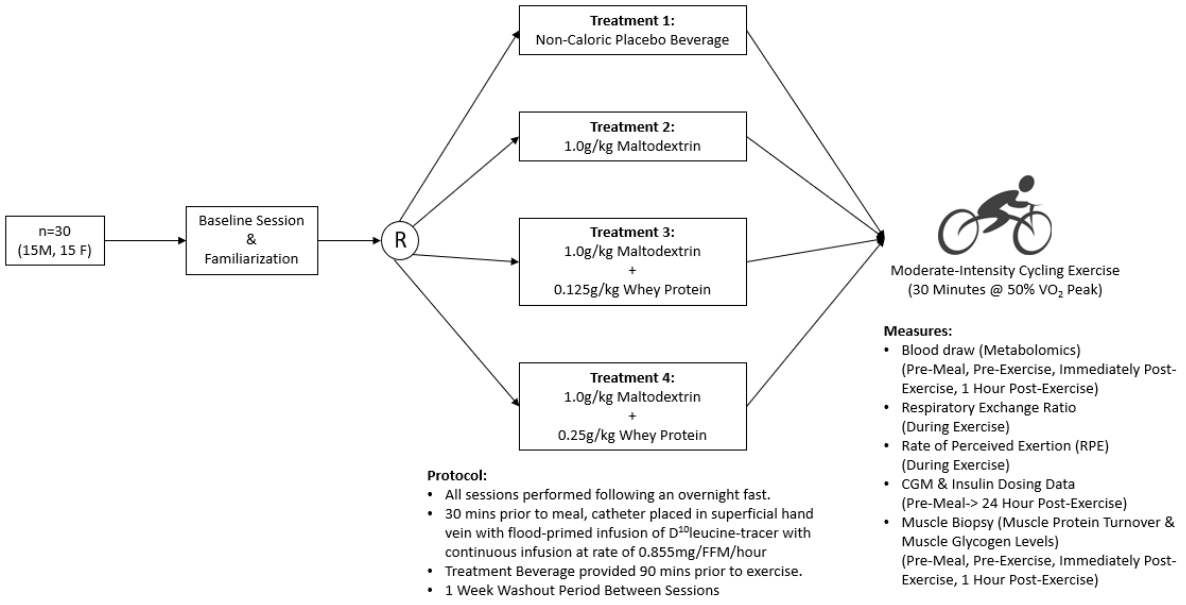


Figure 6.1. Proposed Study Design to Assess the Effects of Pre-Exercise Carbohydrate + Protein vs Carbohydrate Alone on Glycemic, Metabolic, and Skeletal Muscle Factors During and Following Exercise

Study Aims

The primary aim of this study is to compare the glycemic response, measured as CGM-based TIR, TBR, & TAR, during and for 24 hours following exercise following each of these pre-exercise nutrition treatments. The secondary aims of this study are to compare the metabolic and skeletal muscle protein synthetic responses to exercise following each nutritional strategy. Tertiary aims of this study would be to compare differences in RPE and RER during exercise following each nutrition treatment.

Proposed Protocol

At their baseline visit, participants height, weight, body composition (DXA), aerobic capacity (VO_2 peak) and baseline glycemia (HbA1c) would be measured and participants would be asked to complete baseline questionnaires (demographics, health history, Diet History

Questionnaire (DHQ III), and Global Physical Activity Questionnaire (GPAQ)). Additionally, a Dexcom G7 continuous glucose monitor would be inserted and initiated and a wearable activity tracker would be provided to participants.

For measurement visits, participants would report to the lab in the morning following an overnight fast. If participants morning glucose levels are below recommendations for safe exercise participation (<90mg/dL) or if they have experienced severe hypoglycemia (≤50 mg/dL) or a hypoglycemic event requiring assistance from another individual within the previous 24 hours, than the visit will be rescheduled.[19] Upon arrival to the lab, if the study provided continuous glucose monitor has fewer than 48 hours of wear time remaining, a new CGM will be placed. To allow for assessment of muscle protein synthesis during and following exercise, a catheter will be placed in a superficial hand vein to allow for initiation of a flood-primed infusion of D^{10}leucine-tracer 30 mins prior to receiving their pre-exercise meal with continuous infusion at a rate of 0.855mg/FFM/hour during exercise and for 1 hour following exercise.[204] Participants would then be provided with a beverage containing either 1.) 1.0g/kg carbohydrate, 2.) 1.0g/kg carbohydrate plus 0.125g/kg whey protein, 3.) 1.0g/kg carbohydrate plus 0.25g/kg whey protein or 4.) a non-caloric placebo beverage 90 minutes prior to participating in 30 minutes of moderate-intensity continuous cycling exercise performed at 50% of VO_2 peak. In alignment with expert consensus guidelines, participants would decrease their insulin dose for this meal by 50% of their usual carbohydrate/insulin ratio.[19]

Blood draws and muscle biopsies of the vastus lateralis will be taken immediately pre-meal, immediately pre-exercise, immediately post-exercise and 1-hour post-exercise from which targeted metabolomic analysis and measures of muscle protein synthesis and muscle glycogen content will be obtained. During exercise, gas exchange will be measured using a metabolic cart

and ratings of perceived exertion will be obtained every 5 minutes and immediately following exercise cessation. Continuous glucose monitoring and insulin dosing data will be provided from the start of the day of the visit through 24-hours post-exercise. To account for other dietary intake throughout the day, participants will be provided with standardized meals to consume at home/work for the rest of their day and participants will be asked to not eat outside of non-study provided food throughout the day.

Significance

This study would provide valuable insight regarding the effects of different doses of pre-exercise protein intake on glycemia during and following exercise compared to carbohydrate alone or a non-nutritive placebo. Additionally, it would provide data on how the protein content of a pre-exercise meal effects substrate utilization, muscle glycogen depletion and muscle protein turnover during exercise which have not been well-studied among people with T1D. Finally, this study provides important qualitative data regarding how pre-exercise protein intake may influence the perception of effort during exercise, an important practical application for athletes with T1D.

6.4.2 Effects of Post-Exercise Protein Intake With or Without Co-Consumption of Carbohydrate Intake on Post-Exercise Glycemia, Recovery and Muscle Metabolism

In Chapters 4, we observed no overall relationship between post-exercise protein intake and post-exercise glycemia, however, a limitation to our analyses was that the protein dose was relatively small in comparison to other studies with similarly long time-periods of observation for glycemia. Additionally, we observed a significant reduction in post-exercise TBR with increasing post-exercise protein intake among female adolescents with T1D. In Chapter 5 we also observed a trend towards reduced TBR with increasing protein intakes

following moderate-intensity continuous exercise among adults with T1D, however, the exercise performed in this study was conducted in the mornings following an overnight fast. Previous studies have shown that exercise performed in the afternoon in a non-fasted state may be more likely to lead to hypoglycemia in the hours following exercise.[16,187] As such, we propose to conduct a randomized controlled trial to examine the effects of different post-exercise nutrition strategies on glycemia overnight following a bout of moderate-intensity continuous cycling exercise among adults with T1D with a recent HbA1c < 13%.

Study Aims

The primary aim of this study would be to compare TIR, TAR, and TBR from consumption of the beverage until the following morning between the four post-exercise nutrition strategies. The secondary aim of this study would be to compare responses between male and female participants following each condition to assess for potential effect measure modification. Tertiary aims of the study would be to evaluate differences between conditions in recovery from exercise (restoration of muscle glycogen and total work performed on isokinetic testing) as well as muscle protein turnover and circulating levels of glucagon, GLP-1 and GIP.

Proposed Protocol

At baseline visits, participants would be familiarized with the study protocol. Following familiarization, participants height, weight, and body composition (DXA) would be measured, and participants would perform a graded exercise test to assess their aerobic capacity (VO_2 peak). Additionally at this visit participants would be asked to complete baseline questionnaires (demographics, health history, Diet History Questionnaire (DHQ III), and Global Physical

Activity Questionnaire (GPAQ)) and a Dexcom G7 continuous glucose monitor would be inserted and initiated.

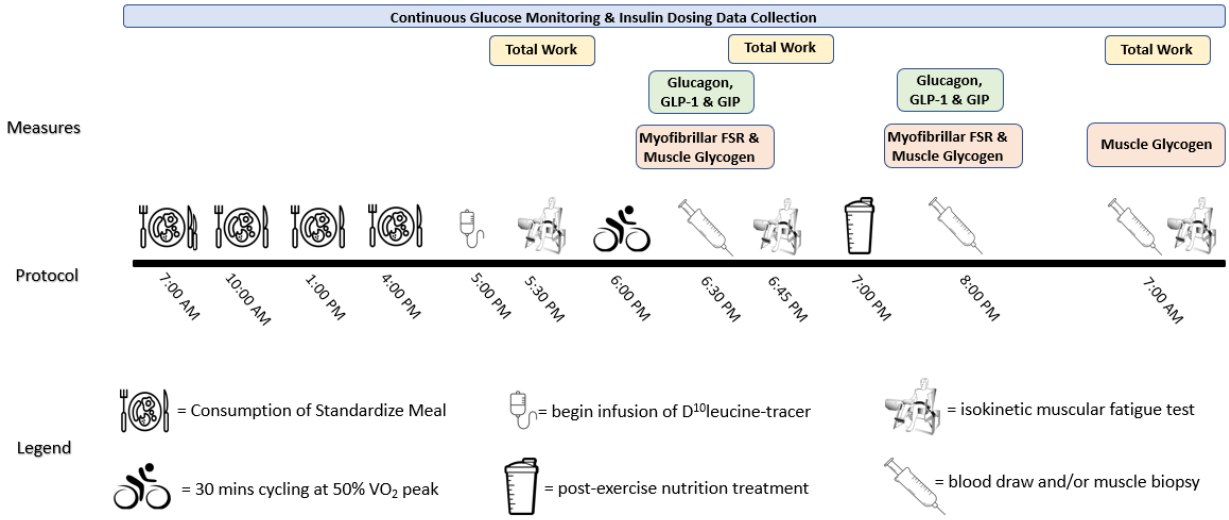


Figure 6.2. Proposed Study Design to Assess the Effects of Different Post-Exercise Nutrition Strategies on Glycemia, Recovery, and Muscle Metabolism Following Afternoon Exercise

An illustration of the proposed study protocol is provided in **Figure 6.2.** We propose providing standardized meals to participants to be consumed throughout the day leading up to exercise bouts at equal intervals. Following consumption of the final pre-exercise meal, a catheter will be placed in a superficial hand vein to allow for initiation of a flood-primed infusion of D10leucine-tracer with continuous infusion at a rate of 0.855mg/FFM/hour throughout exercise and for 1 hour following exercise to allow for measurement of muscle protein turnover.[204] Thirty minutes prior to exercise, participants would perform an isokinetic muscular fatigue test consisting of 30 reciprocal maximal contractions of knee flexors and extensors at 180^{o} s^{-1}.[205] Following the isokinetic muscular fatigue test, participants would participate in 30 minutes of moderate intensity cycling exercise at 50% of VO_2 peak. Immediately following

exercise, a blood draw and muscle biopsy of the vastus lateralis would be collected, followed by another isokinetic muscular fatigue test.

Following these tests, participants would consume one of four post-exercise nutrition beverages: 1.) A non-caloric placebo beverage, 2.) 50g of maltodextrin, 3.) 25g maltodextrin + 25g whey protein or 4.) 50g whey protein. An additional blood draw and muscle biopsy would be collected for an hour following the consumption of the post-exercise nutrition beverage. Participants would report back to the lab the following morning where they would then complete another isokinetic muscular fatigue test and an additional muscle biopsy to allow for comparison of muscular recovery from the previous days exercise bout and to compare differences in muscle glycogen content following each nutritional strategy. Participants would experience each treatment condition separated by at least 7 days.

Significance

This study would provide valuable insights into the role of post-exercise protein intake, with or without carbohydrate, on overnight glycemia as well as the contributions of potential hormonal (glucagon, GLP-1, GIP) and muscular (muscle glycogen content) mediators of the effect. It would also us to further explore whether the response to post-exercise protein intake may differ by sex as was observed in our Chapter 4 analyses. Finally, this study would provide insights into the effects of different nutritional strategies on post-exercise muscle protein turnover and recovery of anaerobic capacity following exercise which have been scarcely evaluated in the setting of type 1 diabetes.

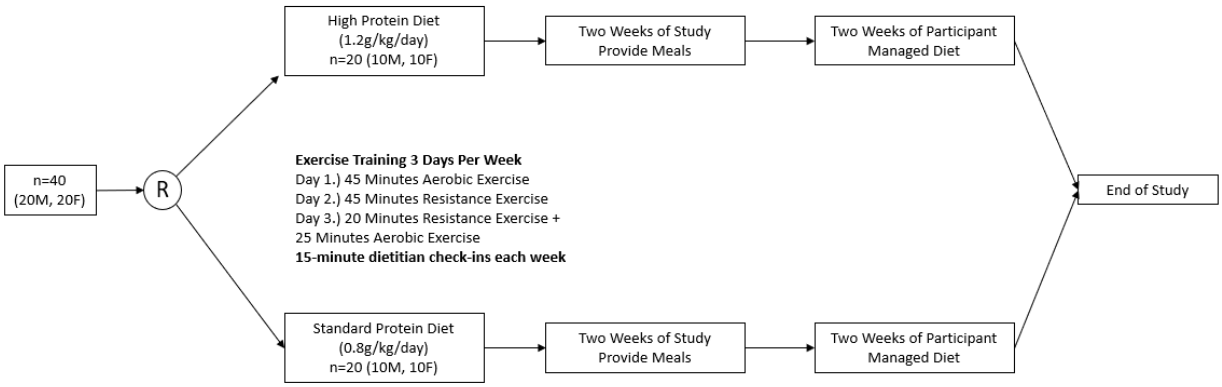
6.4.3 Effects of Following a High Protein Diet on Glycemic Management During 4-Weeks of Exercise Training Among Young Adults with T1D and Overweight & Obesity

In Chapter 4, we identified that following a high protein diet, in line with sports nutrition recommendations of 1.2-2.0g/kg/day, provided benefits in post-exercise glycemic control among adolescents with T1D. These benefits were found to be greatest among female adolescents, those with overweight or obesity and those on multiple daily insulin injections. Additionally, in Chapter 5, we observed that the association between elevated protein intakes and post-exercise glycemia may differ by the type or intensity of the exercise performed. Following a higher protein diet has also been associated with numerous adaptive benefits such as improvements in muscular strength and reductions in soreness, however, these adaptive benefits haven't been confirmed among people with T1D.[120,159] In the following study, we would propose conducting a randomized controlled diet trial among young adults with T1D who also have overweight or obesity and a recent HbA1c < 13% to examine the effects of a high protein diet combined with exercise training on glycemic management, muscular strength and aerobic capacity.

Study Aims

The primary aim of this study would be to evaluate the effects of following a high protein diet on glycemic management across all four weeks and during each two-week time period separately, as well as comparing differences in glycemic response between diet assignments following each type of exercise session. The secondary aims of this study would be to evaluate the effect of diet assignment on weight, lower body strength and aerobic capacity, compare average reports of muscular soreness by diet assignment, and to assess the feasibility, acceptability and barriers to implementing a high protein diet among people with T1D.

Proposed Protocol



Measures:
Baseline Physical Measures: Anthropometrics, Hemoglobin A1c, Body Composition (DXA), Aerobic Capacity (VO_2 Peak), Lower Body Strength (Leg Press 1RM)
Other Baseline Measures: Demographics, Health History, Global Physical Activity Questionnaire (GPAQ), Diet History Questionnaire (DHQ-III)
Measures Collected Throughout Study: Continuous Glucose Monitoring, Insulin Dosing, Accelerometry, Muscle Soreness Visual Analogue Scale (VAS)
Measures Collected In First Two Weeks: Diet Feasibility & Acceptability Questionnaire
Measures Collected in Last Two Weeks: Three 24-Hour Recalls, Diet Feasibility & Acceptability Questionnaire
Endline Measures: Anthropometrics, Hemoglobin A1c, Body Composition (DXA), Aerobic Capacity (VO_2 Peak), Lower Body Strength (Leg Press 1RM)

Figure 6.3. Proposed Study Design to Assess the Effects of Following a High Protein Diet on Glycemic Management During 4-Weeks of Exercise Training Among Young Adults with T1D

Figure 6.3 provides an illustration of the proposed study protocol. Participants would report to the study site for a baseline visit where they would have their height, weight, HbA1c, body composition (DXA), lower body strength (Leg Press One Repetition Maximum (1RM)), and aerobic capacity (VO_2 peak) assessed. Participants would also be asked to complete baseline questionnaires (demographics, health history, GPAQ, DHQ-III) and would have a Dexcom G7 CGM inserted and initiated.

Following their baseline visit, participants would be randomized to either a high protein diet (1.2g/kg/day) or standard protein diet (0.8g/kg/day). For the first two weeks of the study, meals would be provided by the study site and participants would be asked to refrain from consuming food outside of their provided meals. Meals would be provided at exercise visits and participants would be asked to bring back their containers and any uneaten food from previous

meals to confirm diet adherence. Participants would be asked to complete a diet feasibility and acceptability questionnaire near the end of this two-week period to gauge participants perspectives on the diet. Following these first two weeks, participants would then be asked to continue to adhere to their assigned diet with dietitian support for two more weeks. During this time, participants would again be asked to complete a diet feasibility and acceptability questionnaire to gauge perspectives, barriers, and facilitators to implementing a high protein or standard protein diet and three, 24 hour diet recalls would be performed to assess adherence to their assigned study diet.

Throughout the study, participants would be asked to visit the study site three times per week to engage in 45 minutes of exercise with coaching and supervision from a member of the study staff, with one day per week consisting of 45 minutes of resistance exercise, one day consisting of 45 minutes of moderate-intensity aerobic exercise, and one day consisting of a combination of resistance and aerobic exercise (20 minutes of resistance exercise + 25 minutes of aerobic exercise). This exercise strategy is based on physical activity guidelines for people with T1D which recommend achieving 150 minutes of MVPA per week, spread across at least 3 days per week, with the incorporation of resistance exercise two days per week. This strategy would also allow for the observation of how glycemia differs during and following three different exercise modalities. Throughout the study, participants will be asked to provide CGM and insulin dosing data and a muscle soreness visual analogue scale will be completed at the beginning of each exercise session to gauge participants perceived level of muscle soreness.

Following completion of the four weeks of diet intervention, participants would complete follow-up measures of weight, body composition (DXA), HbA1c, aerobic capacity, and lower body strength. Participants would also be asked to complete a short interview to share their

feedback regarding their perceptions of the feasibility of the study diets and exercise training as well as barriers and facilitators to glycemic management related to their assigned study diet or the exercise intervention.

Significance

This study would provide valuable evidence regarding the effects of following a high protein diet, both in a controlled setting and in a free-living environment, on glycemic management with exercise training. It would also allow for the exploration of differing effects of a high protein diet on glycemia by exercise type and sex. Additionally, this study would help to determine whether adaptive benefits associated with a high protein diet in healthy populations are translatable to a population with T1D. Finally, this study would also provide valuable qualitative data on participants perceptions of the acceptability and feasibility of a incorporating a high protein that would inform whether a high protein diet is practical to implement in a real-world setting as well as specific barriers and facilitators to incorporating a high protein diet that will inform the development of future interventions.

6.4.4 Additional Research Needs

The proposed studies outlined in the preceding sections provide initial steps which can build off this dissertation research while also beginning to incorporate other measures of health, fitness and adiposity, but additional work is needed. Mechanistic studies, utilizing mouse models of type 1 diabetes, can help to identify the various mechanisms through which protein influences glycemia which would guide the development of more tailored intervention studies. Additionally, there is a need for strategies to assist people with T1D with weight management which is complicated by the challenges of managing with exercise and caloric restriction

glycemia.[6] High protein diets, when combined with a hypocaloric diet, have been to promote favorable changes in body composition with increased fat loss and greater retention of muscle mass in healthy populations and those with T2D.[125,159,173,206] As this dissertation research has demonstrated that a high protein diet may improve the glycemic response to exercise for people with T1D, it may be an ideal nutritional strategy to support people with T1D in promoting weight management. Finally, future studies should aim to incorporate mixed methods as the qualitative feedback of people living with T1D is essential in informing the practical application of research findings in clinical practice and also in identifying future research needs and directions that may more adequately address the various barriers and facilitators to improving diet and glycemic management.

6.5. Significance and Implications

Exercise-related dysglycemia, specifically hypoglycemia, is a major concern of people with T1D, preventing many from achieving recommended levels of physical activity.[5,7] Previous work with the FLEX study has shown that, over a 7-day period, nearly 80% of adolescents had experienced at least 1 case of clinical hypoglycemia and over 50% had experienced clinically severe hypoglycemia.[202] Expert consensus guidelines on carbohydrate consumption and insulin dosing strategies to mitigate the risk of hypoglycemia were published in 2017 and have been helpful in providing strategies that can begin to help people with T1D manage their glycemia around exercise.[19] A limitation to these strategies, however, is that some individuals with T1D report that making adjustments to insulin dosing can require significant trial and error and having to consume carbohydrate to prevent hypoglycemia can create a feeling of futility around exercise when weight management is a motivator for exercising.[6,207] This feedback highlights the need to

continue exploring nutrition strategies which may help to combat exercise-related dysglycemia that are also easily implementable and may also support other exercise-related health goals.

The findings of this body of work are significant for both its ability to inform exercise nutrition guidelines for people with T1D, but also because it brings together the fields of sports nutrition and diabetes care to identify nutrition strategies that may improve both the glycemic and adaptive response to exercise among people with T1D. As very few studies have examined the role of protein intake on glycemia in the context of exercise, this dissertation provides valuable data which will inform future studies which can more robustly investigate the potential effectiveness and feasibility of this sports nutrition strategy in addressing adverse glycemic excursions among people living with T1D. Importantly, it also provides helpful context as to how the factors such as sex, weight status, insulin regimen, and exercise modality may influence the relationship between protein intake and exercise-related glycemia as well as potential scenarios in which a high protein diet may complicate glycemic management, such as among adolescents without overweight or obesity in which we observed elevated TBR among those who reported consuming a high protein diet. Finally, the strategies investigated in these studies have been shown to support a range of adaptive benefits ranging from improvements in recovery time and muscle soreness to enhancements in athletic performance and body composition.[120,195,208] While more work is needed in this area, these studies suggest that following sports nutrition recommendations for dietary protein intake has potential for improving both the safety and benefits of exercise for people with T1D, supporting people with T1D in engaging in more regular physical activity.

6.6. Closing Remarks

With the majority of people with T1D reporting not achieving the recommended level of physical activity, it's important that we continue to pursue strategies to support them in overcoming the barriers which prevent them from experiencing the health benefits of a physically active lifestyle.[4,69] As the fear of hypoglycemia is a leading barrier to exercise participation across ages of individuals with T1D, preventing hypoglycemia should remain a priority in the development of nutrition guidelines to support exercise among people with T1D, however, it is also important that researchers and clinicians keep in mind the goals and motivators that inspire people to participate in exercise.[5,7,8] As with people without diabetes, there are many reasons that people with T1D may choose to exercise, such as supporting weight management, enhancing athletic performance or cardiovascular fitness, managing stress, or improving mental health. To fully support people with T1D in being more physically active, we should strive to not only address the barriers they face, but also the goals and motivators that encourage them to be more active. In forming exercise nutrition guidelines, attention should be given to not only the role of diet in glycemic management, but also the myriad of other potential health benefits that may be experienced with thoughtful consideration of how diet may augment the physiologic and adaptive response to exercise for people with T1D. By bringing together sports nutrition and diabetes care nutrition approaches, we can provide more comprehensive care to people with T1D and empower them to not only optimize their glycemic, but also their physical fitness and well-being.

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