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Pre-exercise protein intake is associated with reduced time in hypoglycaemia among adolescents with type 1 diabetes

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Abstract

Aim: Secondary analyses were conducted from a randomized trial of an adaptive behavioural intervention to assess the relationship between protein intake (g and g/kg) consumed within 4 h before moderate-to-vigorous physical activity (MVPA) bouts and glycaemia during and following MVPA bouts among adolescents with type 1 diabetes (T1D).

Materials and Methods: Adolescents (n = 112) with T1D, 14.5 (13.8, 15.7) years of age and 36.6% overweight/obese, provided measures of glycaemia using continuous glucose monitoring [percentage of time above range (>180 mg/dl), time in range (70-180 mg/dl), time below range (TBR; <70 mg/dl)], self-reported physical activity (previous day physical activity recalls), and 24 h dietary recall data at baseline and 6 months post-intervention. Mixed effects regression models adjusted for design (randomization assignment, study site), demographic, clinical, anthropometric, dietary, physical activity and timing covariates estimated the association between pre-exercise protein intake on percentage of time above range, time in range and TBR during and following MVPA.

Results: Pre-exercise protein intakes of 10-19.9 g and >20 g were associated with an absolute reduction of −4.41% ($p = .04$) and −4.83% ($p = .02$) TBR during physical activity compared with those who did not consume protein before MVPA. Similarly, relative protein intakes of 0.125-0.249 g/kg and ≥0.25 g/kg were associated with −5.38% ($p = .01$) and −4.32% ($p = .03$) absolute reductions in TBR during physical activity. We did not observe a significant association between protein intake and measures of glycaemia following bouts of MVPA.

Conclusions: Among adolescents with T1D, a dose of ≥10 g or ≥0.125 g/kg of protein within 4 h before MVPA may promote reduced time in hypoglycaemia during, but not following, physical activity.

KEYWORDS
dietary intervention, exercise intervention, glycaemic control, type 1 diabetes

1 | INTRODUCTION

For adolescents living with type 1 diabetes (T1D), regular physical activity improves glycated haemoglobin (HbA1c), cardiorespiratory fitness, body mass index (BMI), insulin sensitivity, lipid profiles and perceived well-being.[1–3] The risk of hypoglycaemia with physical activity, however, is a major barrier to regular participation in physical activity among this population.[4,5] 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 before physical activity have been established[6]; however, less is known about the role of pre-exercise protein intake on glycaemia during or following physical activity. Sports nutrition guidelines suggest that adding protein to a carbohydrate containing meal within 4 h before exercise may promote improved recovery from a proceeding exercise bout by promoting increased muscle protein synthesis and reduced muscle protein breakdown during exercise.[7–10] Among adolescents and children with T1D, protein ingestion has also been shown to induce a mild, prolonged increase in glycaemia lasting up to 5 h following a meal, with one study showing higher glucose concentrations as much as 12 h following fat and protein-rich meals among adolescents with T1D.[11–14] In theory, this hyperglycaemic effect of protein, if consumed before exercise as suggested by sports nutrition guidelines, may help to mitigate declining glycaemia during and following physical activity among adolescents with T1D.

To our knowledge, only one study has examined the effect of pre-exercise protein intake on glycaemia among adolescents with T1D.[15] In this study, the authors monitored blood glucose levels during 60 min of moderate-intensity cycling exercise following either a protein supplemented breakfast consumed 2 h before exercise, a standard breakfast with a carbohydrate supplement 15 min before exercise, or a standardized breakfast with a placebo drink before exercise. They found that consumption of a protein-supplemented breakfast 2 h before exercise was equally effective in reducing the risk of hypoglycaemia during exercise compared with a standardized breakfast, which was supplemented with a carbohydrate beverage consumed 15 min before exercise (n = 10).[15] To our knowledge, however, no previous studies have investigated whether pre-exercise protein intake may improve glycaemia in the hours following physical activity. This is an important gap in the literature as the risk of hypoglycaemia is elevated for up to 24 h following activity in people with T1D.[16] As the effects of protein on glycaemia have been shown to persist for at least 5 h and possibly as long as 12 h,[11,14] it is 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 h before a bout of moderate-to-vigorous physical activity (MVPA) on the percentage of time spent in recommended glucose range (TIR, 70-180 mg/dl), percentage of time spent above range (TAR, >180 mg/dl) and percentage time spent below range (TBR, <70 mg/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.

2 | MATERIALS AND METHODS

2.1 | Study design

Data were analysed from the Flexible Lifestyles Empowering Change (FLEX) study[17,18] (1UC4DK101132-01), which is a randomized controlled trial of a behavioural intervention aimed at improving glycaemia, and 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 5 January 2014 to 4 April 2016, 258 adolescents between the ages of 13 and 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 behaviours. 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.[17,18]

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, glycaemic, 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.

2.3 | Measures

2.3.1 | Demographics and health history

Participants completed demographic and health history questionnaires at baseline, 6 months and 18 months following their baseline visit.

TABLE 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%)
4-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)
Glycaemia	
No personal CGM use in past 30 days (n = 102)	71 (69.6%)
Baseline HbA1c (%)	9.3 (8.5, 9.9)
Percentage time in range (n = 106)	36.5 ± 13.7
Percentage time below range (n = 106)	2.1 (0.4, 5.6)
Percentage time above range (n = 106)	59.5 ± 16.1
Diet	
Daily caloric intake (kcal)	1721.0 ± 560.1
Percentage of daily calories from protein	16.0 ± 3.5
Percentage of daily calories from carbohydrate	49.0 ± 7.7
Percentage of daily calories from fat	36.2 ± 6.4
Daily fibre intake (g)	13.1 (10.1, 18.1)
Physical activity (n = 108)	
Meet ADA guidelines of ≥60 min 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)

Note: 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.
Abbreviations: ADA, American Diabetes Association; BMI, body mass index; CGM, continuous glucose monitor; FLEX, flexible lifestyles empowering change; HbA1c, glycated haemoglobin; MVPA, moderate-to-vigorous physical activity.

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).

2.3.2 | Physical activity

Two previous day physical activity records (PDPAR) were collected in conjunction with the 24 h dietary recalls over the phone. The previously validated PDPAR[19,20] divides the day into 30-min 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 min or more)'. Each activity and perception of effort was matched to a corresponding metabolic equivalent of task (MET) value utilizing the compendium of physical activities,[21] as detailed by Weston et al.[20] From these records, bouts of MVPA were defined as ≥30 min of physical activity at a MET value of ≥3 METs.

2.3.3 | 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 metre (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 h after insertion, pre-meal and before bed. The TIR (70-180 mg/dl), TAR (>180 mg/dl) and TBR (<70 mg/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 06.30 h the following morning (Aim 2) utilizing consensus report definitions of TIR, TAR and TBR.[22] As the minimal reportable duration of a bout of activity with the PDPAR[19,23] is 30 min and previous research on the effects of protein intake on glycaemia among adolescents with T1D has shown hyperglycaemic effects lasting at least 5 h,[11–14] these analyses were restricted to observations with at least 30 min of CGM data during bouts of MVPA and at least 5 h of CGM data following those bouts. An example timeline of exposures and outcomes is provided in Figure 1.

2.3.4 | Dietary intake

Two unannounced 24 h 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, Multiple Prinicipal Investigators EMD and SRS), using the Nutrient Data System for Research software and the multiple pass interviewing method.[24,25] Protein intake consumed within 4 h before 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 >3 SDs above the mean (>71.6 g) were excluded as potential outliers. Furthermore, to account for the glycaemic effect of

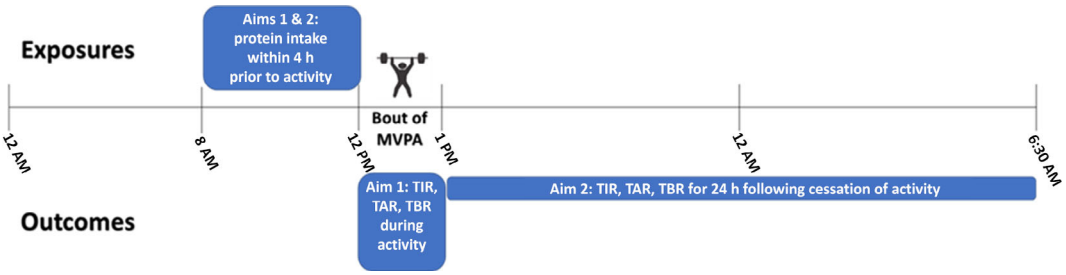

FIGURE 1 Timeline of exposures and outcomes relative to a bout of MVPA using multiple measurements (baseline and 6 months). MVPA, moderate-to-vigorous physical activity; TAR, time above range; TIR, time in range; TBR, time below range.

carbohydrate and bolus insulin levels, pre-exercise carbohydrate intake (g) was considered as a potential covariate in the Aim 1 analyses and daily carbohydrate (g) intake was considered as a potential covariate in the Aim 2 analyses.

2.3.5 | Anthropometrics and 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 fibreglass or steel tape measure, respectively. Height and weight measurements were also used to determine BMI. These measures were used to estimate percentage bodyfat using validated age, race and sex-specific equations.[26] Estimated percentage body fat was considered as a potential covariate in our statistical models.

2.4 | Statistical analysis

All statistical analyses were performed using SAS 9.4 (SAS Institute). Observations with incomplete dietary, physical activity, continuous glucose monitoring or covariate data were excluded in these post-hoc analyses as detailed in Figure 2. Potential sources of selection bias were explored by comparing exposure, covariate and baseline glycaemic 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 h prior to a bout of MVPA and TIR, TBR, TAR during a bout of MVPA (Aim 1) and until 06.30 h the morning following a bout of MVPA (Aim 2). The estimated effect of protein intake (g and g/kg bodyweight) on glycaemia was assessed utilizing a categorical variable to account for non-linearity. Categories for grams of protein intake were defined as <10, 10-19.9 and ≥20 g of protein. Categories for g/kg were defined as <12.5, 0.125-0.25 and ≥0.25 g/kg bodyweight. In both sets of analyses, non-consumers of protein during the 4 h preceding the exercise bout were chosen as a reference group. These categories were based on sports nutrition recommendations, which suggest a protein intake of 0.25 g/kg or an absolute dose of 20-40 g as an optimal level to promote positive adaptations to exercise.[9,10] To assess whether smaller doses may be effective in promoting improved exercise-related glycaemia, 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 kg), body composition (estimated body fat percentage), physical activity [average bout METs, bout duration (min), bout volume (MET-min), other daily physical activity (MET-min)], 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.

2.4.1 | Evaluation of potential effect measure modification by exercise intensity

Previous studies have shown that higher intensity physical activities, such as high-intensity interval training or resistance training, may lead to glycaemic responses that differ from more moderate-intensity activity.[27–29] As such, stratified analyses were performed to explore potential effect measure modification by exercise intensity. To do so, we stratified our analyses using a dichotomous variable (1 = vigorous intensity, 0 = moderate intensity). These analytic models adjusted for the same models as our primary models with the exception that bout duration (min) was substituted for bout volume (MET-min) as analyses were being stratified by intensity, which is based on MET values of activity.

3 | RESULTS

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 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

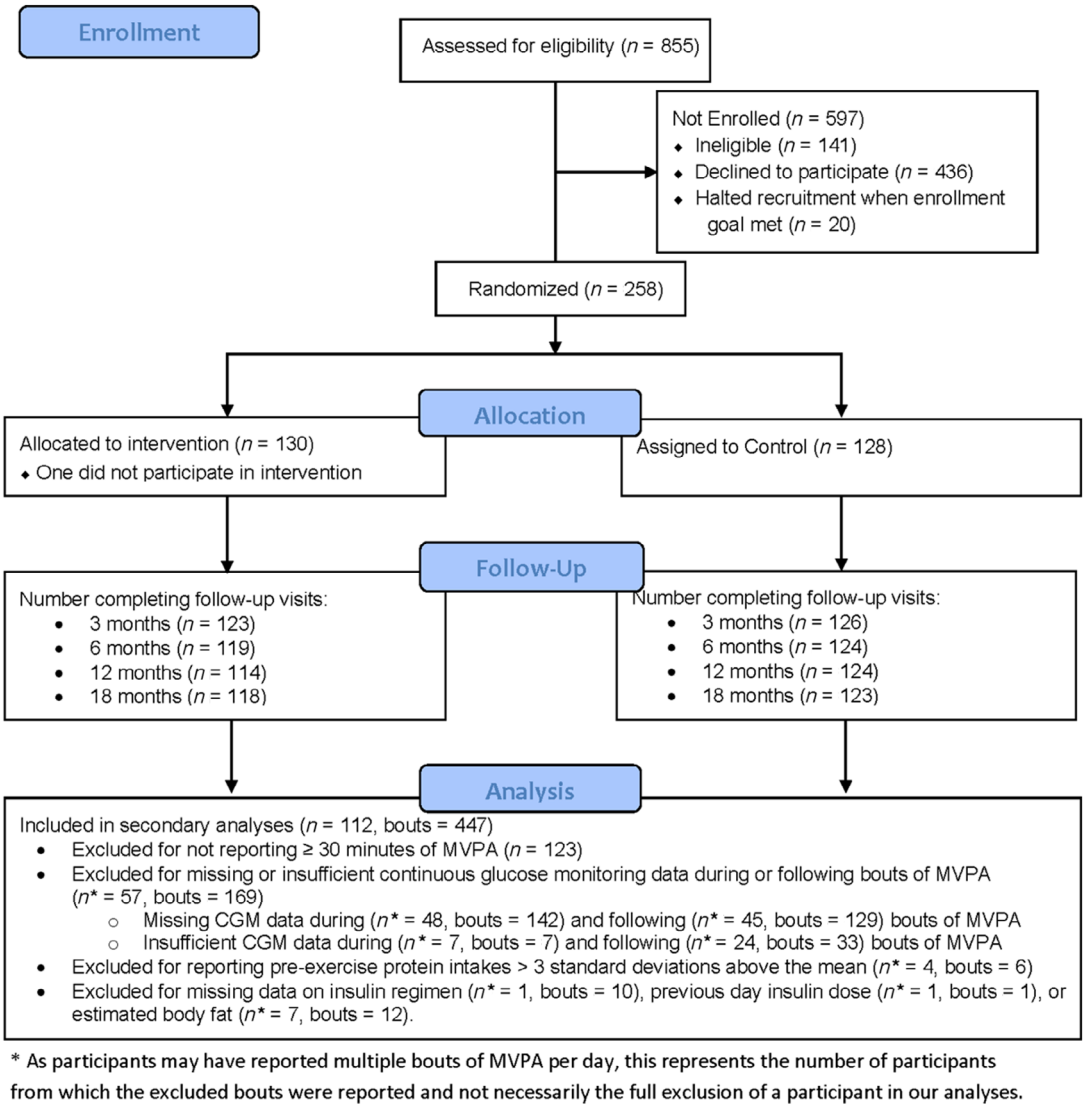

FIGURE 2 Consort flow diagram for secondary analyses of the FLEX randomized trial. CGM, continuous glucose monitor; FLEX, flexible lifestyles empowering change; MVPA, moderate-to-vigorous physical activity.

nutrient intake, weight, BMI Z-score, baseline HbA1c, or any other covariate included in our statistical models.

3.2 | Baseline characteristics

Baseline characteristics of participants included in our analyses are provided in Table 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.

3.3 | Effects of absolute and relative protein intake within 4 h prior to a bout of moderate-to-vigorous physical activity on glycaemia during physical activity

The median protein intake within 4 h prior to a bout of MVPA was 14.0 (IQR: 5.0, 26.3) g or 0.23 (IQR: 0.08, 0.41) g/kg of bodyweight. The mean TIR, TAR and TBR during MVPA bouts was 34.50% ± 41.64%, 62.37% ± 43.53% and 3.13% ± 13.99%, respectively. We observed that protein intakes of 10-19.9 g and ≥20 g compared with 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 0.125-0.249 g/kg and ≥0.25 g/kg compared with 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.

TABLE 2 Results of mixed effects regression models estimating the association between protein intake (g) consumed within 4 h before a bout of moderate-to-vigorous physical activity and glycaemia 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 g protein (bouts = 99)	0.80%	.90	(−11.80%, 13.39%)	−1.06%	.62	(−5.33%, 3.21%)	0.26%	.97	(−12.82%, 13.34%)
10-19.9 g protein (bouts = 122)	−1.45%	.81	(−13.59%, 10.69%)	**−5.08%**	**.02**	**(−9.19%, −0.97%)**	6.15%	.34	(−6.47%, 18.76%)
≥20 g protein (bouts = 159)	2.36%	.69	(−9.30%, 14.02%)	**−5.86%**	**<.01**	**(−9.80%, −1.91%)**	3.56%	.56	(−8.56%, 15.67%)
Fully adjusted models[a]									
<10 g protein (bouts = 99)	2.58%	.69	(−10.22%, 15.39%)	−0.46%	.33	(−6.84%, 2.30%)	−2.03%	.76	(−15.30%, 11.23%)
10-19.9 g protein (bouts = 122)	0.22%	.97	(−12.10%, 12.54%)	**−4.41%**	**.04**	**(−8.57%, −0.25%)**	3.85%	.55	(−8.93%, 16.62%)
≥20 g protein (bouts = 159)	1.19%	.85	(−11.17%, 13.54%)	**−4.83%**	**.02**	**(−9.00%, −0.66%)**	3.74%	.56	(−9.07%, 16.53%)

Note: Bold values are those that are statistically significant.
Abbreviations: MET, metabolic equivalent of task; MVPA, moderate-to-vigorous physical activity; TAR, time above range; TIR, time in range; TBR, time below range.
[a]Estimates are adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/min, pre-exercise carbohydrate intake and hours until midnight. Reference group = no protein intake within 4 h prior to MVPA bouts (bouts = 67).

TABLE 3 Results of mixed effects regression models estimating the association between relative protein intake (g/kg) consumed within 4 h before a bout of moderate-to-vigorous physical activity and glycaemia 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									
<12.5 g/kg (bouts = 72)	4.33%	.53	(−9.15%, 17.81%)	0.91%	.69	(−3.63%, 5.45%)	−5.28%	.46	(−19.23%, 8.67%)
0.125-0.249 g/kg (bouts = 102)	−0.90%	.89	(−13.45%, 11.65%)	**−5.69%**	**.01**	**(−9.92%, −1.47%)**	6.57%	.32	(−6.42%, 19.58%)
≥0.25 g/kg (bouts = 206)	0.12%	.98	(−11.14%, 11.38%)	**−5.56%**	**<.01**	**(−9.35%, −1.77%)**	5.35%	.37	(−6.32%, 17.02%)
Fully adjusted models[a]									
<12.5 g/kg (bouts = 72)	6.27%	.37	(−7.41%, 19.95%)	1.60%	.49	(−2.98%, 6.19%)	−7.80%	.28	(−21.93%, 6.33%)
0.125-0.249 g/kg (bouts = 102)	0.70%	.91	(−11.98%, 13.38%)	**−5.38%**	**.01**	**(−9.63%, −1.13%)**	4.72%	.48	(−8.39%, 17.82%)
≥0.25 g/kg (bouts = 206)	−0.71%	.91	(−12.48%, 11.07%)	**−4.32%**	**.03**	**(−8.27%, −0.38%)**	4.94%	.42	(−7.23%, 17.10%)

Note: Bold values are those that are statistically significant.
Abbreviations: MET, metabolic equivalent of task; MVPA, moderate-to-vigorous physical activity; TAR, time above range; TIR, time in range; TBR, time below range.
[a]Estimates are adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/min, pre-exercise carbohydrate intake, and hours until midnight. Reference group = no protein intake within 4 h before MVPA bouts (bouts = 67).

TABLE 4 Results of mixed effects regression models estimating the association between protein intake (g) within 4 h prior to a bout of moderate-to-vigorous physical activity and glycaemia following cessation of physical activity until 06.30 h 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 g protein (bouts = 99)	1.20%	.74	(−6.00%, 8.39%)	−0.29%	.81	(−2.68%, 2.10%)	−0.71%	.85	(−8.30%, 6.89%)
10-19.9 g protein (bouts = 122)	1.04%	.77	(−5.90%,7.98%)	−1.13%	.33	(−3.44%, 1.17%)	0.30%	.94	(−7.03%, 7.63%)
≥20 g protein (bouts = 159)	1.45%	.67	(5.22%, 8.12%)	0.28%	.8	(−1.93%, 2.49%)	−1.41%	.69	(−8.45%, 5.63%)
Fully adjusted models[a]									
<10 g protein (bouts = 99)	1.56%	.67	(−5.75%, 8.87%)	−0.50%	.68	(−2.92%, 1.93%)	−0.92%	.81	(−8.64%, 6.80%)
10-19.9 g protein (bouts = 122)	1.72%	.63	(−5.31%, 8.74%)	−1.16%	.33	(−3.49%, 1.17%)	−0.37%	.92	(−7.79%, 7.06%)
≥20 g protein (bouts = 159)	0.54%	.88	(−6.32%, 7.40%)	0.30%	.80	(−1.98%, 2.57%)	−0.52%	.89	(−7.76%, 6.72%)

Abbreviations: MET, metabolic equivalent of task; MVPA, moderate-to-vigorous physical activity; TAR, time above range; TIR, time in range; TBR, time below range.

[a]Estimates are adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/min, daily carbohydrate intake, post-activity protein intake and hours until midnight. Reference group = no protein intake within 4 h prior to MVPA bouts (bouts = 67).

3.4 | Effects of absolute and relative protein intake within 4 hours prior to a bout of moderate-to-vigorous physical activity on glycaemia from cessation of physical activity until 06.30 hours the following morning

The mean duration of time from the end of MVPA bouts until 06.30 h the following morning was 15.56 ± 3.89 h and the mean TIR, TAR and TBR during this time was 40.80% ± 24.84%, 55.58% ± 28.00% and 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 06.30 h the following morning. Estimated associations ranged from −0.24% to 1.72% ($p > .52$), −1.16% to 0.30% ($p > .33$) and −1.47% to 0.50% ($p > .68$) for TIR, TBR and TAR, respectively across absolute and relative intakes of protein pre-exercise.

3.5 | Effect measure modification of the estimated effect of pre-exercise protein intake on time below range during bouts of moderate-to-vigorous physical activity by exercise intensity

The results of analyses stratified by exercise intensity for the estimated effects of relative and absolute pre-exercise protein intake on glycaemia during and following exercise are reported in Tables 1-4. Stratified analyses found that protein intakes of 10-19.9 g and ≥20 g were associated with an −6.69% (95% CI: −11.59%, −1.78%) and −8.15% (95% CI: −13.01%, 3.29%) reduction in TBR for moderate-intensity bouts of activity, respectively, but not for vigorous intensity bouts, 1.78% (95% CI: −6.60%, 10.16%) and 2.18% (95% CI: −6.43%, 10.79%), respectively. Similarly, protein intakes of 0.125-0.25 g/kg and ≥0.25 g/kg was associated with an −8.23% (95% CI: −13.15%, −3.29%) and −7.06% (95% CI: −11.68%, −2.46%) reductions in TBR during exercise for moderate-intensity bouts, respectively, but not vigorous intensity, 1.97% (95% CI: −6.82%, 10.75%) and 1.76% (95% CI: −6.31%, 9.91%). No associations were observed between pre-exercise protein intake and glycaemia following bouts of physical activity for either moderate or vigorous activity.

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 glycaemia during and following exercise among adolescents with T1D. It was hypothesized that elevated protein intake within the 4 h prior to MVPA bouts would be associated with improved TIR and reduced TBR during and following exercise. The results of this study showed that consumption of at least 10 g or 0.125 g/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

TABLE 5 Results of mixed effects regression models estimating the association between relative protein intake (g/kg) within 4 h prior to a bout of moderate-to-vigorous physical activity and glycaemia following cessation of physical activity until 06.30 h 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									
<12.5 g/kg (bouts = 72)	0.91%	.81	(−6.76%, 8.59%)	−0.69%	.59	(−3.25%, 1.86%)	0.02%	1.00	(−8.08%, 8.12%)
0.125-0.249 g/kg (bouts = 102)	−0.85%	.81	(−8.01%, 6.32%)	−0.22%	.86	(−2.61%, 2.17%)	1.27%	.74	(−6.30%, 8.83%)
≥0.25 g/kg (bouts = 206)	2.49%	.45	(−3.95%, 8.93%)	−0.24%	.83	(−2.38%, 1.91%)	−1.97%	.57	(−8.78%, 4.83%)
Fully adjusted models[a]									
<12.5 g/kg (bouts = 72)	1.11%	.78	(−6.69%, 8.91%)	−0.86%	.51	(−3.46%, 1.73%)	−0.05%	.99	(−8.29%, 8.18%)
0.125-0.249 g/kg (bouts = 102)	−0.24%	.95	(−7.47%, 7.00%)	−0.07%	.96	(−2.47%, 2.34%)	0.50%	.9	(−7.14%, 8.14%)
≥0.25 g/kg (bouts = 206)	2.13%	.52	(−4.48%, 8.74%)	−0.42%	.71	(−2.62%, 1.78%)	−1.47%	.68	(−8.45%, 5.51%)

Abbreviations: MET, metabolic equivalent of task; MVPA, moderate-to-vigorous physical activity; TAR, time above range; TIR, time in range; TBR, time below range.

[a]Estimates are adjusted for intervention group, study site, age, sex, insulin regimen, previous day insulin dose (units/kg), estimated body fat percentage, bout MET/min, daily carbohydrate intake, post-activity protein intake and hours until midnight. Reference group = no protein intake within 4 h prior to MVPA bouts (bouts = 67).

association was observed between pre-exercise protein intake and glycaemia following exercise. In addition, in stratified analyses, we observed that the benefits of protein intake on glycaemia were observed only during moderate-intensity bouts of physical activity, which may reflect differing glycaemic trajectories following more high-intensity physical activity; however, more research is needed to clarify the role of exercise intensity has on the effect of pre-exercise protein intake on exercise-related glycaemia.[27–29]

These findings are in agreement with the findings of Dube et al. who observed that, among adolescents with T1D, consuming a protein supplemented breakfast 2 h before exercise was equally effective at preventing hypoglycaemia during exercise compared with a standard breakfast that was followed by the consumption of a carbohydrate beverage 15 min before exercise.[15] While the size of the reduction in TBR may appear relatively small (4.32%-5.69% or ~2.59-3.41 min), 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.[22] As such, these findings represent a clinically significant decrease in TBR during physical activity. While previous studies have shown that the hyperglycaemic effect of protein intake among adolescents with T1D may persist for 5 h or longer,[11,13,14] we did not observe an association between pre-exercise protein intake and glycaemia following MVPA. It is also important to note that, in healthy populations, consuming protein before exercise has been suggested to have potential benefits for promoting recovery or reducing fatigue during exercise among healthy populations.[8,30–32] While such effects have not been tested among people with T1D, it is possible that consuming protein before exercise may be a promising strategy to assist people with T1D in improving both the safety and benefits of exercise.

As with all studies, this study has several limitations. First, self-reported measures of dietary intake are prone to under-reporting because of recall and social desirability biases[33]; however, the use of a multiple pass method for 24 h dietary intake data, as was used in the FLEX study, has been shown to minimize the effects of these biases in dietary intake data.[25,34] Furthermore, MVPA is often over-reported among adolescents compared with accelerometry,[35] 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 ($r = 0.77$, $p < .01$) and identification of MVPA bouts on a previous day (0.63, $p < .01$).[20,36] Furthermore, the use of interviewers to administer recalls of physical activity has been shown provide a more reliable measurement of MVPA compared with self-administered methods.[37] In addition, the lack of time-stamped insulin dosing data for these analyses limits are the ability to understand the role of insulin-dosing behaviours on the observed associations. By controlling for carbohydrate intake in these analyses we hoped to account partially 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 hypoglycaemia.

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 glycaemia during and following physical activity, which begin to address an important gap in the literature and start bridging sports nutrition and diabetes care guidelines.

Randomized controlled trials are needed to establish whether a causal relationship exists between pre-exercise protein intake and glycaemia during exercise and the hours thereafter among adolescents and adults with T1D. As fear of hypoglycaemia is the 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 glycaemia and to inform dietary guidelines to support safe participation in exercise for those living with T1D. In addition, 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 that may promote both enhanced glycaemic management and positive adaptive benefits with exercise among people living with T1D.

5 | PROTOCOL

The FLEX study is registered on clinicaltrials.gov, NCT01286350, and a detailed description of the design,[17] main results[18,38] and secondary analyses[39–42] of the FLEX study have been previously published.

AUTHOR CONTRIBUTIONS

FRM conceptualized the aims and approach of this paper with review and approval of EMD, JLC, AESR, SRS and KRE. Analyses were performed by FRM with consultation by senior statistician JLC. Preparation of the manuscript, figures and tables was performed by FRM with review, editing and approval from all co-authors.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15438.

DATA AVAILABILITY STATEMENT

The data in this study are openly available in the NIDDK Data Repository at doi:10.58020/235v-4k70.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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