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Full Title:

Post-exercise glycemic control in type 1 diabetes is associated with residual β-cell function

Short Title:

Type 1 diabetes: C-peptide vs post-exercise CGM

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Abstract**:**

Objective

To investigate the impact of residual β-cell function on continuous glucose monitor (CGM) outcomes following acute exercise in people with Type 1 diabetes.

Research Design and Methods

Thirty participants with type 1 diabetes for ≥3 years were recruited. Firstly, participants wore a blinded CGM for 7 days of free-living data capture. Secondly, a 3 hour mixed meal test, assessed stimulated C-peptide and glucagon. Peak C-peptide was used to allocate participants into undetectable ($Cpep_{und}$ <3 pmol/L), low ($Cpep_{low}$ 3–200 pmol/L) or high C-peptide groups ($Cpep_{high}$ >200 pmol/L). Finally, participants completed 45 minutes of incline treadmill walking at 60%VO_{2peak} followed by a further 48 hours' CGM capture.

Results

CGM parameters were comparable across groups during the free-living observation week. In the 12 (12hr) and 24 hours (24hr) post-exercise periods the $Cpep_{high}$ group had significantly greater amount of time spent with glucose 3.9-10 mmol/L (12hr: 73.5±27.6%, 24hr: 76.3±19.2%) compared to $Cpep_{low}$ (12hr: 43.6±26.1%, p=0.027, 24hr: 52.3±25.0%, p=0.067) or $Cpep_{und}$ (40.6±17.0%, p=0.010, 24hr: 51.3±22.3%, p=0.041). Time spent in hyperglycemia (12hr and 24hr glucose >10 and >13.9 mmol/L, p<0.05) and glycemic variability (12hr and 24hr SD, p<0.01) were significantly lower in the $Cpep_{high}$ group compared to $Cpep_{und}$ and $Cpep_{low}$. Change in CGM outcomes from pre to 24hr post-exercise was divergent: $Cpep_{und}$ and $Cpep_{low}$ experienced worsening (glucose 3.9-10 mmol/L: -9.1% and -16.2% respectively), with $Cpep_{high}$ experiencing improvement (+12.1%)(p=0.017).

Conclusions

Residual β-cell function may partially explain the inter-individual variation in the acute glycemic benefits of exercise in individuals with type 1 diabetes. Quantifying C-peptide could aid in providing personalized and targeted support for exercising patients.

Introduction:

Individuals with type 1 diabetes are encouraged to regularly engage in physical activity (PA) and exercise because of many health benefits, such as reduced cardiovascular risk factors and improvements in physical fitness [1]. However, exercise can cause disruption to maintaining euglycemia, particularly when causing hypoglycemia, and can be complex to manage [2]. This may explain the lower PA levels within the type 1 diabetes population compared to the general public [3].

One major obstacle to providing exercise support to people with type 1 diabetes is a high inter-individual variability in the blood glucose responses to exercise [2]. Even within tightly controlled research studies that have adopted a strict inclusion criteria, recruited a homogenous cohort of participants, have standardized insulin and dietary intake and used continuous glucose monitoring (CGM) to stabilise pre-trial glucose, a large unexplained inter-individual variability in the acute glycemic responses to exercise remains [4-7]. This is despite a high intra-individual reproducibility under repeated conditions [4,5]. Indeed, outside of formal research, both clinical observations and feedback from patient support groups report potential for both an improvement and detrimental impact of regular exercise on HbA1c. Wide-ranging challenges in successfully avoiding hypoglycemia persist, despite advancement and availability of supportive strategies including CGM and patient education.

Recent research has shown that even in long duration type 1 diabetes, β-cell function – as measured by C-peptide – can persist. There is some disparity within the evidence regarding the prevalence of residual β-cell function within the type 1 diabetes population, but it is estimated that between 35 and 80% of participants have detectable β-cell function at >5 years post-diagnosis [8,9]. Moreover, it is estimated that 8-16% of individuals diagnosed with type 1 diabetes as an adult have a relatively high C-peptide level, above the threshold found in the Diabetes Control and Complications Trial (DCCT: >200 pmol/L) to have some clinical benefits [10], compared to 5-6% of individuals with childhood onset of diabetes [8,9,11].

Evidence from recently diagnosed individuals and after islet transplantation, when consequently C-peptide levels are relatively high, demonstrates that as residual β-cell function declines, CGM parameters such as time in euglycemia (time in range 3.9-10

mmol/L) and CV% worsen [12,13]. A recent paper by Rickels et al.[14] demonstrated that individuals with short-duration type 1 diabetes and very high stimulated C-peptide (>400 pmol/L) had greater time in euglycemia at rest compared to negative, low (17-200 pmol/L) and intermediate (200-400 pmol/L) C-peptide groupings. How this translates to people with established, longer-duration type 1 diabetes and around exercise, is unclear. Potentially, diminished but functioning β-cells may convey some level of intrinsic glucose regulation that offers benefits under an intense metabolic stressor (including increased metabolic rate, carbohydrate oxidation and insulin sensitivity) such as exercise. Moreover, it can be hypothesized that β-cell function is associated with CGM outcomes explaining (at least in part) inter-individual variability in the exercise response. This information could be valuable for provision of targeted exercise support, based on C-peptide status.

This study examined the impact of residual β-cell function on CGM outcomes after a bout of aerobic exercise in people with type 1 diabetes. We hypothesized that individuals with greater C-peptide will have increased amount of time with an interstitial glucose in euglycemia (3.9-10 mmol/L) – the primary outcome.

Research Design and Methods:

Participants

Eligibility criteria comprised a clinical diagnosis of type 1 diabetes (primary osmotic symptoms, weight loss, hyperglycemia, ketosis, insulin initiation at diagnosis); age 18-65 years with diabetes duration ≥3 years at enrolment; HbA1c <86 mmol/mol (10.0%); absence of diabetes-related complications apart from retinopathy; and stable Multiple Daily Injection (MDI) or Continuous Subcutaneous Insulin Infusion (CSII) regimen without changes over the preceding 6 months. All participants provided written informed consent and this study was approved by the local National Health Service Research Ethics Committee (code:16/NE/0192, registry:ISRCTN50072340).

Sample Size

Sample size estimation was calculated using available C-peptide and CGM data previously conducted by our group [13]. Using percentage time in range 3.9–10 mmol/L during a 5-day CGM capture from islet transplant recipients with stimulated C-peptide >200 pmol/L (71±21%) and <150 pmol/L (45±16%). With an estimated difference of at least 10% in the primary outcome, a sample of 10 participants per group would be needed to test the null hypothesis that mean time within range (3.9-10.0 mmol/L) of all groups is equal with a probability of 0.8. Type 1 error associated with this calculation is 0.05.

Participant identification and recruitment

Potential participants with ≥3 years duration were first identified using a home Urine C-peptide to Creatinine Ratio (UCPCR) kit [15]. Three years was used to allow a clear gap from the approximate 2 year point often referred to as the ‘honey moon’ [16]. UCPCR results were used to preliminarily allocate participants in one of three UCPCR groupings: undetectable (<0.001), low (0.001-0.19) and high (≥0.2 nmol/mmol). Supplement figure 1 has a schematic of the study recruitment numbers and protocol.

Visit 1: Free-living Observational CGM Week

Participants attended the Newcastle NIHR Clinical Research Facility (CRF) for insertion of a blinded CGM unit (Enlite® sensor with iPro^{TM}2 Professional CGM

Medtronic Diabetes, Medtronic MiniMed, USA). During the observational free-living week, patients self-recorded insulin dosages and capillary blood glucose (CBG) concentrations. CBG were recorded ≥4 times per day for calibration purposes with sensor data retrospectively processed using CareLink software (Medtronic Diabetes). If a day's CGM recording, from midnight to midnight, failed any of the Carelink optimal data thresholds (Valid calibrations, MAD%, Correlations)[17] or had missing data greater than 15 minutes segments, data from throughout that day were deemed sub-optimal and not used. If the iPro^{TM}2 failed to collect 4 valid days of data the testing process was repeated.

Visit 2: Mixed Meal Tolerance Test

Participants attended the CRF at ~8.30am after an overnight fast and a cannula was inserted into an antecubital vein. Individuals were instructed to maintain their normal basal insulin regimen. A mixed meal tolerance test (MMTT) protocol was used, with participants given 240 ml of Fortisip (Nutricia, Trowbridge, UK; 360kcal, 14.4g protein, 13.92g fat and 44.16g carbohydrate) to drink within 2 minutes [18]. Blood samples were drawn at baseline and every 30 minutes up to and including 180 minutes. Samples were centrifuged with plasma and serum stored at -80 °C in the Newcastle Biobank facility.

Visit 3: Health screening and Maximum Exercise Test

Participant height, weight (Seca 220 stadiometer / Seca 889 scales, Seca, Germany) and medical history were taken. Participants underwent a modified 12-lead resting and exercising electrocardiogram (ECG) to screen for cardiac abnormalities.

A maximal graded walking treadmill (Valiant 2 CPET, Lode, Groningen, Netherlands) test (Bruce protocol [19]) was performed to determine peak oxygen uptake (VO_{2peak}) and peak heart rate. Glycemic strategy was managed as per the guidance of Riddell et al.[2].

Visit 4: Main trial exercise bout

Prior to the submaximal exercise phase, participants attended the CRF 24-48 hours before the final testing visit, to have a CGM inserted. Individuals arrived at the exercise lab at ~8.30am after an overnight fast, having been instructed to maintain their normal basal insulin regimen. If participants had a hypoglycemic event overnight prior to the

study visit, the visit was rearranged, while if participants awoke with blood glucose >10 mmol/L they were instructed to have a small corrective bolus of insulin upon waking (≤2 units). A carbohydrate (CHO) snack (Belvita, Mondelēz International, USA), providing 204kcal of which 31g CHO, was consumed and participants remained rested for 20 minutes. Target CBG was >7 mmol/L for the duration of the exercise, with participants given 10g of carbohydrate if CBG fell below this level. Participants walked at 60% VO_{2peak} for 45 minutes at a comfortable stride length (7.15±3.58% gradient at 5.09±0.28kph). Individual treadmill speed and gradient was calculated using VO_2, speed, and gradient data from the preliminary exercise test [20].Heart rate and expired air were captured and analysed throughout (Metalyzer® 3B-R3 CPET, Cortex, Leipzig, Germany), with gradient adjusted at 10 and 30 minutes if VO_2 was >10% different than target VO_2. Upon completion of the exercise, participants rested for 60 minutes before being discharged from the laboratory. For the 48 hours following the exercise bout free-living interstitial glucose responses were captured and participants recorded CBG.

Blood Sample analysis

Samples from Visit 2 were transported to Exeter Clinical Laboratories for analysis of serum C-peptide, glucagon and auto-antibodies. C-peptide was analysed using a direct electrochemiluminescence immunoassay (E170 analyser, Roche Diagnostics, Mannheim, Germany) as described elsewhere [21]. Lower limit of detection was 3.3 pmol/l with a reported intra- and inter-assay coefficient of variation of 3.3% and 4.5% [22]. Individual's peak serum C-peptide recorded during the MMTT was used to confirm which C-peptide groups participants were sorted into; undetectable ($Cpep_{und}$) <3 pmol/L, low ($Cpep_{low}$) 3–200 pmol/L and high ($Cpep_{high}$) >200 pmol/L. The high C-peptide grouping was based upon the clinically significant threshold found in the DCCT [10], while the low C-peptide threshold was based upon the lower limit of detection of the assay. Serum glucagon was measured using a Glucagon ELISA (Mercodia AB, Uppsala, Sweden) on the Dynex DS2 automated platform (Dynex Technologics, Worthing, U.K) with a lower limit of detection of 1.5 pmol/L.

Auto-antibody analysis was performed using ELISA assays (RSR Ltd., Cardiff, UK) on the DS2 automated platform (Dynex Technologics, Worthing, U.K) as previously reported [23]. The cut-offs for positivity were: ≥7.5 U/mL (IA-2); ≥11 U/mL (GAD65);

≥65 U/mL (ZnT8) if aged < 30 years or ≥9.1 U/mL if aged >30 years. Positive result defined as above the 97.5th centile of 1,559 control participants without diabetes [23].

Statistical and data analysis

Data are presented as mean±standard deviation throughout unless otherwise stated with statistical significance set at $p<0.05$. The primary outcome was amount of time with an interstitial glucose in euglycemia (3.9-10 mmol/L) in the 24 hours post-exercise. Secondary outcomes were euglycemia at 12 hours, and glycemic variability (standard deviation (SD) and coefficient of variance (CV)), time spent in hypoglycemia and time spent in hyperglycemia in the 12 and 24 hours post-exercise. CGM ranges were defined as 3.9-10 mmol/L (euglycemia), <3.9 mmol/L (hypoglycemia 1), <3.0 mmol/L (hypoglycemia 2), >10 mmol/L (hyperglycemia 1), >13.9 mmol/L (hyperglycemia 2) as recommended by international consensus [24]. CV was calculated as SD divided by mean glucose.

Statistically significant differences between the means of $Cpep_{und}$, $Cpep_{low}$ and $Cpep_{high}$ were determined by one-way ANOVA with Tukey post-hoc analysis. Data were assessed for normality and outliers by Shapiro-Wilk test and boxplots, with skewed data assessed by Kruskal-Wallis H test. Pearson product-moment or Spearman's rank-order correlation were used to determine the strength and direction of a linear relationship between peak MMTT serum C-peptide and glucagon vs CGM data. GraphPad Prism 8.0.1 (San Diego, USA) and IBM SPSS Statistics (version 24, IBM, Armonk NY) software package were used to analyse the data.

Results:

Three participants who were initially recruited with a 'Low' UCPCR, subsequently demonstrated an undetectable peak serum C-peptide. Additionally, two participants with 'Undetectable' UCPCR subsequently showed 'Low' C-peptide positivity during the MMTT.

Participants were allocated into three groups according to MMTT peak serum C-peptide. Demographic and MMTT group data are shown in Table 1. Age, HbA1c, BMI, insulin and VO_{2peak} were comparable between groups. However, the $Cpep_{high}$ group had significantly higher age of diagnosis and shorter duration of diabetes than the $Cpep_{und}$. Although C-peptide metrics differed between groups (in keeping with the study design), MMTT glucagon values were comparable. Fasting glucose was comparable at baseline of the MMTT, with the $Cpep_{high}$ group having significantly lower peak and delta change compared to the $Cpep_{und}$.

***** INSERT TABLE 1 HERE *****

Observational week

Data was collected for an average 5.1±0.96 days, with no differences between groups (p=0.730). During the observational week, there were no differences between the C-peptide groups in time spent in euglycemia (Figure 1A), hypoglycemia or hyperglycemia, mean glucose, SD or CV. MMTT C-peptide and glucagon values did not predict any CGM outcomes during the observational week (p>0.05)(Table 2).

Laboratory phase - Exercise bout

On average, participants exercised at 59.4±4.1% of their VO_{2peak}, with no differences between the C-peptide groups (p=0.542). The $Cpep_{und}$ group had higher CBG on arrival ($Cpep_{und}$ 9.83±2.17, $Cpep_{low}$ 7.96±3.11, $Cpep_{high}$ 7.25±1.52 mmol/L, p=0.045), pre ($Cpep_{und}$ 11.42±2.76, $Cpep_{low}$ 9.37±1.61, $Cpep_{high}$ 8.30±1.14 mmol/L, p=0.007) and post-exercise ($Cpep_{und}$ 13.00±4.38, $Cpep_{low}$ 9.26±4.37, $Cpep_{high}$ 9.00±2.83 mmol/L, p=0.048), as well on leaving the laboratory at 1 hour post-exercise ($Cpep_{und}$ 13.34±3.21, $Cpep_{low}$ 11.23±3.86, $Cpep_{high}$ 9.32±2.58 mmol/L, p=0.029), compared to the $Cpep_{high}$ but not the $Cpep_{low}$ group. There were no incidences of hypoglycemia

within the laboratory phase of the study, either during the exercise or throughout the 60 minute post-exercise recovery. Six participants (1 $Cpep_{und}$, 2 $Cpep_{low}$ and 3 $Cpep_{high}$) were given 10g of additional carbohydrates during the exercise bout as their blood glucose had dropped below 7 mmol/L.

Post exercise

Twelve and 24 hour post-exercise interstitial glucose responses are presented in Figure 1B+C and Table 2. The $Cpep_{high}$ group spent 73.51±27.64% of the 12 hours post-exercise in euglycemia, compared to 43.58±26.07% for $Cpep_{low}$ ($p=0.027$) and 40.61±16.97% for $Cpep_{und}$ ($p=0.010$)(Figure 1.B). The $Cpep_{high}$ group also had significantly less time spent in hyperglycemia (Categories 1 and 2), lower mean glucose and SD compared to $Cpep_{low}$ and $Cpep_{und}$ ($p<0.05$). No difference existed between groups for time spent with CGM glucose <3.9 mmol/L ($p=0.766$) or <3.0 mmol/L ($p=0.370$), although notably mean time with CGM <3.0 mmol/L was zero in the $Cpep_{high}$ group.

***** INSERT FIGURE 1 HERE *****

Similar patterns were observed in the interstitial glucose response in the 24 hours post-exercise period, with $Cpep_{high}$ having higher time in euglycemia (76.25±19.16%) than $Cpep_{und}$ (51.33±22.26%, $p=0.041$), although not statistically higher than $Cpep_{low}$ (52.31±24.98%, $p=0.067$)(Figure 1.C). $Cpep_{high}$ had significantly lower amount of time spent in hyperglycemia and reduced measures of GV compared to both $Cpep_{low}$ and $Cpep_{und}$.

In the 24 to 48 hours following the exercise bout, the effects were largely lost with only time spent >13.9 mmol/L and SD significantly lower in the $Cpep_{high}$ group compared to $Cpep_{und}$ and $Cpep_{low}$ (Table 2. Figure 1.D).

Peak stimulated glucagon was comparable across groups and did not predict time in hypoglycemia or any CGM measure post exercise ($p>0.05$).

Delta change (Δ) in interstitial glucose parameters from the observational week to 24 hours post-exercise showed significant correlations between peak C-peptide and time in euglycemia (Figure 2.A), time spent >10 mmol/L (Figure 2.C), time spent >13.9 mmol/L and measures of glucose variability (Figure 2.D).

***** INSERT FIGURE 2 HERE *****

Participants with higher C-peptide had increased percentage time in euglycemia in the 24 hours following the exercise bout compared to their free-living observational week (Δ12.11±21.54%), whereas individuals with low (Δ-16±24%, p=0.018) or undetectable (Δ-9.1±18%, p=0.073) C-peptide had reduced time in euglycemia compared to the observational week.

Auto-antibody status

Individual auto-antibody positivity status is displayed in Supplement table 1. Nine of the 30 participants were auto-antibody negative, including 2 participants within the $Cpep_{high}$ group (duration of diabetes: 17 and 20 years, peak C-peptide 532 and 1170 pmol/L, respectively). To reduce the possibility of misdiagnoses of type 2 or monogenic diabetes influencing the results, we reassessed the data excluding these participants.

Between group differences within the first 12 hours post exercise mirrored those seen within the whole group analysis, with time spent in euglycemia significantly higher for $Cpep_{high}$ than $Cpep_{low}$ and $Cpep_{und}$ (p=0.023). When extended out to 24 hours the trends persisted, with clinically relevant, but not statistically significant mean differences ($Cpep_{und}$ 51.33±22.26, $Cpep_{low}$ 52.31±24.98, $Cpep_{high}$ 73.35±19.88%, p=0.093). Furthermore, the same relationships between C-peptide and Δ from the observational week to 24 hours post-exercise for euglycemia (r=0.473, p=0.041), <3.9 mmol/L (r=-0.192, p=0.328), >10 mmol/L (r=-0.355, p=0.064) and CV (r=-0.432, p=0.022) exist.

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Conclusions:

We investigated how residual β-cell function impacts CGM outcomes following exercise in people with type 1 diabetes. We show in the cohort studied, that under free-living conditions, time in euglycemia is comparable despite wide-ranging residual β-cell function. Regardless, and for the first time, we demonstrate that individuals with type 1 diabetes with higher residual β-cell function (stimulated C-peptide >200 pmol/L) displayed a substantially greater amount of time spent in euglycemia in the hours following a bout of moderate intensity exercise. Furthermore, we show divergence in the impact of exercise on glycemic profiles, with high residual C-peptide associated with improved control compared with pre-exercise free-living conditions and low/absent C-peptide associated with worsened control following exercise.

Results from the baseline observational free-living CGM data are similar to Rickels et al.[14]. While they demonstrated that individuals with C-peptide >400 pmol/L spent greater time in euglycemia under free-living conditions, there was no differences between the negative, low (17-200 pmol/L) and what they have defined as intermediate (200-400 pmol/L) groups. Participants in the current study were all attending a single diabetes center. They had mainly good to moderate HbA1c, similar insulin treatment, with access to the same clinical management and education. These factors likely contributed to the comparable time in euglycemia, despite different levels of C-peptide, under these stable free-living conditions.

Our primary findings that individuals with higher C-peptide had substantially increased time in euglycemia post-exercise compared to lower C-peptide individuals, in addition to the clear divergence in whether there is a positive or negative impact of exercise on CGM parameters depending on residual C-peptide status have not previously been reported. These findings were despite the cohort having comparable free-living CGM outcomes and HbA1c. We hypothesize that the endogenous insulin secretion within the $Cpep_{high}$ group combined with increased insulin sensitivity following the exercise bout attenuated high blood glucose excursions. Indeed, the results from the MMTT demonstrated an attenuated glucose response within the high C-peptide group. Exercise can independently increase glucose uptake into the skeletal muscles via the redistribution of GLUT4 glucose transporters to the cell membrane [25]. A single bout

of endurance exercise also increases insulin's action [26], with sensitivity to insulin persisting up to 48 hours post exercise [27]. These mechanisms may contribute to the difficulties in maintaining time in euglycemia after exercise in those with low C-peptide, while enhancing the beneficial impact of endogenous insulin secretion within higher C-peptide individuals.

Authors from previous secondary analysis of glycemic control during and after exercise have postulated that insulin resistance may play a role in the inter-individual variability [28]. As a longer duration of diabetes is associated with increased insulin resistance [29], and the $Cpep_{high}$ group had a lower mean duration, this study cannot rule out the role insulin resistance plays in post-exercise glycemic control. However, it is important to note that the BMI (25.22±3.73 kg/m^2), total daily insulin dose (41.77±23.40 units) and dose per kg (0.55±0.24 units/kg/day) were comparable across groups, and were not high enough to indicate insulin resistance.

Avoidance of hypoglycemia, in everyday life as well as during and after exercise, is of central importance for people with type 1 diabetes. A wide range of methods, including nutritional and insulin adjustments have been reported and discussed, yet difficulties in maintaining euglycemia around exercise are prevalent [2]. Previous studies have reported that preserved β-cell function was associated with reduced self-reported hypoglycemia [30-31], however neither this study or previous have seen time spent in hypoglycemia as measured by CGM influenced by C-peptide [14]. In the current study, time spent in hypoglycemia (<3.9 and 3 mmol/L) in the post exercise period was ≥2-fold less in the $Cpep_{high}$ group, which may be clinically meaningful although not statistically different. Future studies should carefully consider how to most meaningfully measure hypoglycemia in free-living conditions, with a combination of CGM and diaries likely to be needed [32].

This study provides further evidence that the paradoxical glucagon secretion in response to oral ingestion is not influenced by C-peptide status, and that peak glucagon measured by these methods does not associate with time spent in hypoglycemia [14,33]. However, recent research demonstrates that during a hyperinsulinemic hypoglycemic clamp, those with persistent β-cell function have residual counter-regulatory responses to hypoglycemia including increased glucagon [34]. Additionally, there is a reduction in biochemical hypoglycemia and an increase in

glucagon response to hypoglycemic clamp in C-peptide positive islet transplant recipients [16]. The α-cell's ability to secrete glucagon in response to hypoglycemia is impaired around diagnosis of type 1 diabetes [35], with further functional losses as duration of diabetes increases [36]. It is hypothesized that functioning β-cells within the islet of Langerhans enable residual α-cell function allowing some hypoglycemia protection, although underlying mechanisms remain unclear [37]. Whether responses to a hyperinsulinemic clamp have significant impact in real world conditions requires studies such as the current one.

To further understand the participants' responses in our study, auto-antibody status was assessed to minimise the possibility of misdiagnosed diabetes impacting the results, despite a large proportion of individuals with type 1 diabetes being auto-antibody negative at this longer duration of the disease [38]. Even in the high C-peptide group, the two auto-antibody negative participants met our inclusion criteria of classical presentation of T1D at diagnosis. When these participants were excluded similar patterns were observed, with residual β-cell function associated with post-exercise CGM outcomes. Moreover, the same positive relationship between C-peptide and the delta change in free-living to 24 hours post-exercise euglycemia exists. Limitations of this study include participants being a single cohort from the same diabetes centre and predominantly being in moderate or good control. While the CGM capture was largely from free-living periods, the exercise bout was laboratory based with carefully managed blood glucose. It thus remains unclear whether results can be generalized to the wider exercising type 1 diabetes population.

Keeping in mind the potential for residual beta-cell function to help stabilize time in euglycemia during and after exercise, future research should explore longer-term exercise and its associations with hypoglycemia. Previous studies have demonstrated that exercise can blunt counter-regulatory responses to subsequent hypoglycemia [39], and conversely, antecedent hypoglycemia can blunt hormone responses to exercise [40]. Potentially, residual beta-cell function may limit the burden of hypoglycemia by preserving some of these counter-regulatory responses to repeated bouts of physiological stress, helping facilitate effective and safe long-term exercise. Investigations into whether residual β-cell function influences the glycemic responses to differing modalities of exercise (i.e. resistance, high intensity intermittent training), as well as under a range of different insulin and nutritional strategies around exercise

(i.e. fasted morning exercise) are warranted. Finally, a large long-term trial is needed to explore if C-peptide predicts HbA1c changes with exercise, as well as to explore further glycemic and cardiovascular outcomes, teasing apart whether reported improvements in diabetes complications are due to glycemic improvements and/or potentially a direct impact of C-peptide upon vasculature.

In conclusion, people with type 1 diabetes who have higher residual beta-cell function show improved time in euglycemia following exercise. C-peptide may be useful in identification of patients most at risk of exercise associated dysglycemia. We show that future exercise research should consider level of C-peptide as a factor that may impact study outcomes.

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Author Contributions:

G.T. recruited participants, designed study, researched data, wrote the manuscript. D.W. designed study, researched data, wrote the manuscript. J.S. recruited participants, designed study, provided clinical cover and reviewed/edited the manuscript. A.B. and A.F. recruited participants, provided clinical cover and reviewed/edited the manuscript. T.M. and R.O. analysed samples and reviewed/edited the manuscript. E.S. reviewed/edited the manuscript. K.S. J.S. and T.C. contributed to data collection and reviewed/edited the manuscript.

Conflict Of Interest Statement: The authors have no conflict of interest to declare

Reference To Prior Publication Of The Study In Abstract Form:

Some of the data from this work were presented as an abstract at the 79th Scientific Sessions of the ADA, San Francisco, 2019.

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Tables:

Table 1. Demographic and MMTT results for each C-peptide grouping. Data indicates mean ± SD.

C-Peptide Grouping	$CPEP_{UND}$	$CPEP_{LOW}$	$CPEP_{HIGH}$	p
N	11	9	10	
Male/Female	5/6	6/3	5/5	
Age (Years)	40.09 ± 11.18 (26 to '58)	38.67 ± 14.73 (25 to 61)	35.80 ± 10.98 (18 to 52)	0.738
Age At Diagnosis (Years)	13.27 ± 4.50 (8.00 to 24)	16.56 ± 8.57 (8.00 to 32.00)	25.10 ± 8.20 * (13.00 to 35.00)	**0.003**
Duration Of Diabetes (Years)	26.82 ± 13.24 (13.00 to 47.00)	21.89 ± 13.34 (9.00 to 44.00)	10.70 ± 6.15 * (3.00 to 20.00)	**0.015**
HbA1c (mmol/mol)	61.64 ± 10.64 (42.00 to 78.00)	58.11 ± 7.11 (51.00 to 74.00)	55.40 ± 8.47 (41.00 to 69.00)	0.297
(%)	7.8 ± 3.1 (6.0 to 9.3)	7.5 ± 2.8 (6.8 to 8.9)	7.2 ± 2.9 (5.9 to 8.5)	
BMI (kg/m^2)	25.65 ± 3.27	24.20 ± 4.13	25.67 ± 4.04	0.259
Daily Insulin (units)	39.93 ± 15.15	47.88 ± 23.21	38.30 ± 31.23	0.242
Insulin units/kg/day	0.54 ± 0.19	0.63 ± 0.25	0.49 ± 0.29	0.332
Method Of Control (MDI/CSII)	5/6	4/5	6/4	
VO_{2peak}	35.61 ± 7.69 (21.05 to 49.00)	43.93 ± 9.03 (31.80 to 58.25)	35.67 ± 10.77 (21.25 to 51.00)	0.194
MIXED MEAL TOLERANCE TEST				
Peak C-Peptide (pmol/L)	0.00 ± 0.00 (0 to 0)	42.00 ± 32.58 * (4 to 83)	671.70 ± 435.15 * † (221 to 1640)	**<0.001**
Median	0.00	53.00	568.50	
$AUC_{0TO180min}$ C-Peptide (pmol/L)	0.00 ± 0.00	6026 ± 4452 *	89459 ± 48095 * †	**<0.001**
Peak Glucagon (pmol/L)	14.04 ± 6.74	18.60 ± 13.49	12.45 ± 4.34	0.802
$AUC_{0TO180min}$ Glucagon (pmol/L)	1557 ± 905.8	2072 ± 1370	1259 ± 674.5	0.252
Pre Glucose (mmol/L)	10.12 ± 3.38	9.55 ± 1.62	8.47 ± 3.15	0.428
Peak Glucose (mmol/L)	21.91 ± 2.75	20.03 ± 2.34	17.74 ± 3.59 *	**0.016**
Δ Pre to Peak Glucose (mmol/L)	11.76 ± 2.77	10.48 ± 2.12	9.27 ± 3.02 *	**0.045**
Auto-Antibody Positivity	6/11	7/9	8/10	

*Brackets indicate ranges. * Significantly different to $Cpep_{und}$ † Significantly different to $Cpep_{low}$*

Table 2. One-way ANOVA results for the CGM outcomes of each C-peptide grouping at different time points. Data is mean ± SD

	Free-living Observational Week				12 Hours Post Exercise				24 Hours Post Exercise			
	$Cpep_{und}$	**$Cpep_{low}$**	**$Cpep_{high}$**	**p**	**$Cpep_{und}$**	**$Cpep_{low}$**	**$Cpep_{high}$**	**p**	**$Cpep_{und}$**	**$Cpep_{low}$**	**$Cpep_{high}$**	**p**
< 3 mmol/L	0.7 ± 1.4	1.3 ± 1.9	0.9 ± 1.2	0.710	0.7 ± 2.4	3.0 ± 8.4	0.0 ± 0.0	0.284	1.3 ± 3.7	5.3 ± 15.4	0.5 ± 1.5	0.773
< 3.9 mmol/L	3.5 ± 3.2	8.7 ± 9.7	5.7 ± 5.4	0.540	3.6 ± 5.1	5.9 ± 9.1	1.9 ± 3.2	0.586	3.2 ± 5.1	9.3 ± 16.2	4.1 ± 9.8	0.471
> 10 mmol/L	36.1 ± 14.7	22.8 ± 10.0	30.2 ± 16.3	0.129	55.8 ± 17.5	50.5 ± 30.3	24.6 ± 27.6*	**0.015**	45.5 ± 23.5	38.4 ± 24.8	19.7 ± 19.6 *	**0.043**
> 13.9 mmol/L	8.8 ± 5.9	4.3 ± 3.4	6.8 ± 8.6	0.206	20.2 ± 15.7	23.6 ± 18.1	2.3 ± 6.0*†	**0.001**	12.0 ± 10.2	19.1 ± 20.9	1.3 ± 3.2*†	**0.001**
Mean	9.1 ± 1.2	7.8 ± 1.3	8.5 ± 1.6	0.149	10.7 ± 1.6	10.7 ± 2.9	8.2 ± 1.6*	**0.006**	9.8 ± 1.7	9.6 ± 3.0	7.7 ± 1.5	0.065
SD	3.2 ± 0.6	3.0 ± 0.6	3.1 ± 0.6	0.604	3.4 ± 1.2	3.7 ± 1.0	2.0 ± 1.0*†	**0.003**	3.0 ± 0.9	3.8 ± 1.0	2.0 ± 0.7*†	**<0.001**
CV (%)	36.7 ± 7.6	38.2 ± 7.3	36.5 ± 6.0	0.848	32.5 ± 11.5	36.8 ± 14.2	24.8 ± 9.9	0.098	31.9 ± 10.8	42.1 ± 15.4	26.2 ± 9.6 †	**0.025**

* *indicates significantly different to* $Cpep_{und}$ † *indicates significantly different to* $Cpep_{low}$

Figure legends:

*Figure 1. Group mean±SD and individual data points for time spent in a euglycemic range 3.9 to 10 mmol/L during (A) the observational free-living week, (B) 12 hours post submaximal exercise bout, (C) 24 hours post submaximal exercise bout, (D) between 24 and 48 hours post submaximal exercise bout. Red circles = $Cpep_{und}$ (n=11), Orange circles = $Cpep_{low}$ (n=9), Green circles = $Cpep_{high}$ (n=10). * indicates significantly different to $Cpep_{und}$, # indicates significantly different to $Cpep_{low}$.*

*Figure 2. Scatter plots displaying linear relationships between peak serum C-peptide vs the delta change in glycemic control measures from the free-living observational week to the 24 hours post exercise (n = 30). (A) Delta change in the percentage of time spent in 3.9 to 10 mmol/L, (B) Delta change in the percentage of time spent <3.9 mmol/L, (C) Delta change in the percentage of time spent >10 mmol/L and (D) Delta change in the CV%. * indicates significant correlation.*

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