Study Title: REBEL – CV study: Does Residual β-cell function and exercise offer synergistic protection against hyperglycaemic induced circulating vasoprotective dysfunction and immune deficiency in type 1 diabetes?

Short Title: REBEL – CV

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All the investigators declare that they have no potential conflicts of interest.

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Synopsis

Study Title	REBEL – CV study: Does Residual β-cell function and exercise offer synergistic protection against hyperglycaemic induced circulating vasoprotective dysfunction and immune deficiency in type 1 diabetes?	
Internal ref. no. / short title	REBEL – CV	
Study Design	Observational Clinical and Exercise Study. Basic science study involving procedures with human participants	
Study Participants	12 adult men and women with type 1 diabetes and undetectable C-peptide 12 adult men and women with type 1 diabetes and micro C-peptide 12 adult men and women with type 1 diabetes and low C-peptide 12 adult men and women with type 1 diabetes and high C-peptide	
Planned Study Period	Total: 18 months	
	Objective	Outcome Measures
Cell culture Endothelial Progenitor Cell study	- Investigate if C-peptide pre-treatment of EPCs protects these angiogenic cells against hyperglycaemia-induced apoptosis and dysfunction, in combination with and in comparison to insulin, in sequentially increasing concentrations to determine whether a dose-response relationship exists - To examine serum offers protection to EPCs against hyperglycaemia-induced apoptosis and dysfunction, comparing individuals with T1D and high residual β-cell function to those with undetectable β-cell function and non-diabetes controls - To explore if serum collected immediately following an exercise bout offers protection to	- EPCs function will be tested by endothelial migration assays, endothelial proliferation assays and endothelial tube formation assays. - The rate of apoptosis will be assessed using Annexin V Apoptosis Detection Kit, apoptosis activation will be assessed by p53 and Caspase 3 antibodies. Intracellular reactive oxygen species (ROS) by CellROX™ Green Assay Kit, and cell migratory ability assessed by CXCR4/7 expression, all measured via flow cytometry.

	EPCs against hyperglycaemia-induced apoptosis and dysfunction compared to pre-exercise serum, and whether this protection is influenced by the individuals residual β-cell function and/or supplementation with C-peptide.	
Immunology Study	- Explore whether individuals with established T1D and differing β-cell function have differing immune profiles and responses. - To investigate whether exercise mobilises and alters the function of immune cells in type 1 diabetes, and whether this is influenced by residual β-cell function	- Peripheral blood mononuclear cells will be stimulated with pancreatic proteins to see how their T cells respond measured by ELIspot - The abundance and activity of white blood cell populations, focusing on Regulatory T cells, will be measured by Flow Cytometry - Autoantibodies and inflammatory cytokines will be measured in the blood

Scientific Summary

A substantial number of people with type 1 diabetes (T1D) have some degree of residual β-cell function[1]. Low levels of endogenous insulin and C-peptide secretion appear to be clinically beneficial, improving glycaemic control[2–6] and protective against diabetes-related complications[2,3,7–9]. However, the mechanisms underlying protection against microvascular complications are unknown, potentially including improved glycaemic control resulting in reduced vascular damage, and/or endogenous insulin/C-peptide secretion having vasoprotective properties.

Residual β-cell function may influence diabetes-related complications through angiogenic cells. Individuals with T1D have reduced circulating counts[10,11], with lower concentrations associated with increased complications and cardiovascular events[10–13]. We recently demonstrated [14], that exercise-induced mobilization of endothelial progenitor cells (EPCs) is attenuated in T1D, compared with controls. Strikingly, we also found those with high β-cell function displayed similar exercise-induced EPC mobilisation to the controls, while those with undetectable C-peptide displayed a near totally blunted response to exercise. *In vitro* studies have shown that C-peptide may be protective against hyperglycaemia-induced dysfunction in vascular endothelial cells[15,16], while acute exercise improves the function of EPCs[17].

In recent years, there is increasing awareness that type 1 diabetes is a heterogenetic disease. Among others, there is vast variation in the age of onset, genetic susceptibility, rates of disease progression, insulin secretory capacity, diabetes complications, glycaemic control, and therapeutic intervention efficacy [18]. Evidence demonstrates diverse immune dysfunction at diagnosis, which is associated with differencing rates of residual β-cell function destruction[19–22]. However, it is unknown whether this immune dysfunction differs in individuals with established type 1 diabetes, and whether distinct endotypes such as β-cell function and autoantibody status can distinguish them.

We will investigate how residual β-cell function may influence angiogenic cells by exploring 1) if pre-treating EPCs with C-peptide is protective against hyperglycaemia, 2) if pre-treating EPCs with serum from individuals with high β-cell function is protective against hyperglycaemia, compared to those with no β-cell function and controls, 3) if post-exercise serum offers protection to EPCs against hyperglycaemia in comparison to pre-exercise serum, and whether this is influenced by participants β-cell function and/or C-peptide supplementation.

We will also explore whether immune deficiency in individuals with established type 1 diabetes differ between individuals with varying β-cell function, both at rest and after exercise. Specifically, we will 1) measure leukocytes populations both *ex-vivo* and after pancreatic antigen stimulation, 2) measure pancreatic autoantibodies in the blood, 3) measure the T-cell response to pancreatic autoantibodies.

Lay Summary

In type 1 diabetes (T1D), an individual's own immune system attacks the cells that create insulin, a hormone that controls blood sugar. Individuals with T1D have different types of autoimmune responses, with more severe responses quickly destroying all of the insulin producing cell. Up to 80% of people with T1D for >3 years do still release small amounts of insulin and C-peptide, a molecule involved in the creation of insulin, from the pancreas.

For people with T1D, exercise can be beneficial, potentially reducing the progression of diabetes-related complications. Creating insulin/C-peptide may also help protect against diabetes complications, although exactly how is currently unknown.

One possible way is through endothelial progenitor cells (EPCs), which circulate in the blood and repair blood vessels; with T1D associated with having lower numbers of these important cells. We have discovered that individuals who no longer produce any insulin/C-peptide have lower resting count and are not able to increase the number of EPCs after exercise, compared to higher counts and exercise-induced increases for those who still produce insulin/C-peptide.

Exercise can also be beneficial for the immune system. Very limited research suggests that beneficial mobilisation of immune cells with exercise is blunted in people with T1D. However it is not known whether having some ability to create insulin/C-peptide influences this. While different immune cell profiles at diagnosis can predict the rate of destruction, it

is unknown whether the profiles differ between individuals with established diabetes and varying levels of insulin/C-peptide.

This study will explore how having some ability to still make insulin/C-peptide in T1D influences how well EPCs work in normal and high glucose conditions and whether this works in combination with exercise, as well as whether the immune response at rest and post-exercise is different between those who create no insulin/C-peptide and those who do.

Research Aims and Objectives:

To explore whether residual β-cell function in T1D, through C-peptide secretion and/or circulating factors, and exercise associated circulating factors protect endothelial progenitor cells (EPC) from hyperglycaemia-induced apoptosis and dysfunction.

- To investigate if C-peptide pre-treatment of EPCs protects against hyperglycaemia-induced apoptosis and dysfunction, in combination and comparison to insulin, in incremental concentrations to determine whether a dose-response relationship exists.

- To examine if circulating factors within serum protect EPCs against hyperglycaemia-induced apoptosis and dysfunction, comparing individuals with T1D and high residual β-cell function to those with undetectable β-cell function and non-diabetes controls.

- To explore if circulating factors within serum collected immediately following a high intensity exercise bout protect EPCs against hyperglycaemia-induced apoptosis and dysfunction in comparison to pre-exercise serum, and whether this protection is influenced by β-cell function and/or C-peptide supplementation.

We hypothesize that C-peptide directly attenuates hyperglycaemia-induced apoptosis and dysfunction in EPCs. Determining whether this is the case and whether these effects are replicated with serum from those with high residual β-cell function could determine an underlying mechanism by which β-cell function protects against diabetes-induced vascular complication.

To explore whether individuals with established T1D and varying residual β-cell functions have different immune profiles and responses, both at rest and post exercise.

- To investigate if leukocytes populations, both ex-vivo and after pancreatic antigen stimulation, differ between individuals with T1D with undetectable, micro, low or high β-cell function and how that compares to non-diabetes controls

- Investigate whether the T-cell response to pancreatic autoantibodies differs between individuals with T1D with undetectable, micro, low or high β-cell function and how that compares to non-diabetes controls

- Measure pancreatic autoantibodies, cytokines and hormones in the blood, comparing individuals with T1D with undetectable, micro, low or high β-cell function and non-diabetes controls

We hypothesize that individuals with established T1D and undetectable β-cell functions will different immune profiles and have greater severity of immune reaction to pancreatic antigen stimulation than those with high β-cell functions. Determining whether this immune profile are heterogenic in established T1D could help immunotherapy research attempting to halt the progression of T1D, as well as research attempting to restore β-cell function.

Methods

Study Methods	**Basic science study involving procedures with human participants** Purchased EPCs will be tested under 3 conditions: 1 -Investigate if C-peptide pre-treatment of EPCs protects against hyperglycaemia-induced apoptosis and dysfunction, in combination and comparison to insulin. 2 - Examine if circulating factors within serum protect EPCs against hyperglycaemia-induced apoptosis and dysfunction, comparing individuals with T1D and high residual β-cell function to those with undetectable β-cell function and non-diabetes controls

	3 - Explore if circulating factors within serum collected immediately following a high intensity exercise bout protect EPCs against hyperglycaemia-induced apoptosis and dysfunction in comparison to pre-exercise serum, and whether this protection is influenced by β-cell function and/or C-peptide supplementation. **Observational study of human participants** Blood sample measures will be taken at rest and post exercise and examined for: 1 – Leukocytes populations, both ex-vivo and after pancreatic antigen stimulation 2 – Whether the T-cell respond to pancreatic autoantibodies 3 - Pancreatic autoantibodies, cytokines and hormones
Planned Study Period	Total: 18 months
Planned Recruitment period	01/03/2022-01/10/2023
Study Setting	Newcastle NIHR Clinical Research Facility Exercise laboratory Newcastle University's Sport and Exercise Science laboratory Newcastle University Diabetes group research laboratory Newcastle University Flow Cytometry Core Facility

Study Design

This study will comprise participants attending two or three occasions. The study is an observational exercise study design using human participants. All participants will attend the laboratory to be screened for eligibility using a medical questionnaire and to provide written informed consent visit (visit 1). Measurements of resting blood pressure, heart rate, body mass, height, peak expiratory flow, body composition, and blood glucose will also be taken. Participants will attend a second visit for a resting blood sample, or a resting blood sample and maximal cardiopulmonary exercise test (CPET) to the limit of tolerance (visit 2). On a separate day, the participants who completed the CPET will also attend a third visit, completing a high intensity interval exercise bout (HIE) with blood samples taken pre and post-exercise (visit 3).

Study Settings

Participants with T1D will attend Newcastle NIHR Clinical Research Facility on two or three occasions, depending if they are eligible for the exercise protocols.

The EPCs experiments will be performed in the Newcastle Diabetes Research group laboratory. The measurements of leucocyte populations, T-cell reactivity and circulating factors within the blood will take place at the Newcastle University's Sport and Exercise Science laboratory, Newcastle University Diabetes group research laboratory and Newcastle University Flow Cytometry Core Facility.

Study Recruitment

Participants will be recruited from the local area using various recruitment strategies that we have used successfully in the past, including Public and Patient Involvement (PPI) groups, local charities, targeted social media advertisement, posters, flyers, and email list and by contacting previous participants who have agreed to be contacted.

Individuals with T1D will be recruited from the Newcastle Diabetes Centre. Individuals who show interest and are assessed as suitable by the Diabetes Centre clinicians and nurses will have their contact details (email or phone number) passed to the research team, after giving verbal consent.

The research team will contact individuals who declared an interest in the study. A screening call will be undertaken, participant information sheets emailed or posted to the individual and the screening and consent visit organised.

Participants

This study will recruit 44 individuals with T1D, who have a range of residual β-cell function, in total.

For the T1D participants, we will attempt to recruit 12 participants per residual β-cell function category, accounting for a 20% dropout rate. These categories will be determined by post-prandial urine C-peptide Creatinine ratio (UCPCR). The UCPCR will be used to assess residual β-cell function. This test has good sensitivity and specificity for identifying C-peptide thresholds[6,23].

- Undetectable C-peptide (<0.001 nmol/mmol)
- Micro C-peptide (0.001 – 0.030 nmol/mmol)
- Low C-peptide (0.030 – 0.100 nmol/mmol)
- High C-peptide (>0.100 nmol/mmol)

We will follow American College of Sports Medicine (ACSM) guidelines on exercise pre-participation screening to exclude those who are at increased risk for adverse exercise-related cardiovascular events (see [24]). Specific eligibility criteria are displayed below:

Inclusion Criteria for individuals with T1D
• Willing and able to provide informed consent • Aged 18 to 50 years old • Clinical diagnosis and classic presentation of T1D (primary osmotic symptoms, weight loss, hyperglycemia, ketosis, insulin initiation at diagnosis) • T1D diagnosis for ≥2 years
Exclusion Criteria for individuals with T1D
• Participation in another research study • Aged >50 years or <18 years • Resting hypertension (≥160 mmHg systolic and/or ≥90 mmHg diastolic) • Respiratory disease with peak respiratory flow <300 l/min • Previous stroke • Pregnancy • Irregular period • Hormonal treatment for menopause • Unable to read and understand the instructions provided in English • Contraindication to venous blood sampling • Unwilling to undertake exercise • Smoker • Unwilling to undertake and send a home urine sampling kit • Current diagnosis of diabetes complications, including nephropathy, retinopathy (apart from non-proliferative diabetic retinopathy) and neuropathy. • Current or previous diagnosis of other autoimmune or chronic diseases, including cardiovascular disease, renal disease and cancer. • HbA1c >70 mmol/mol

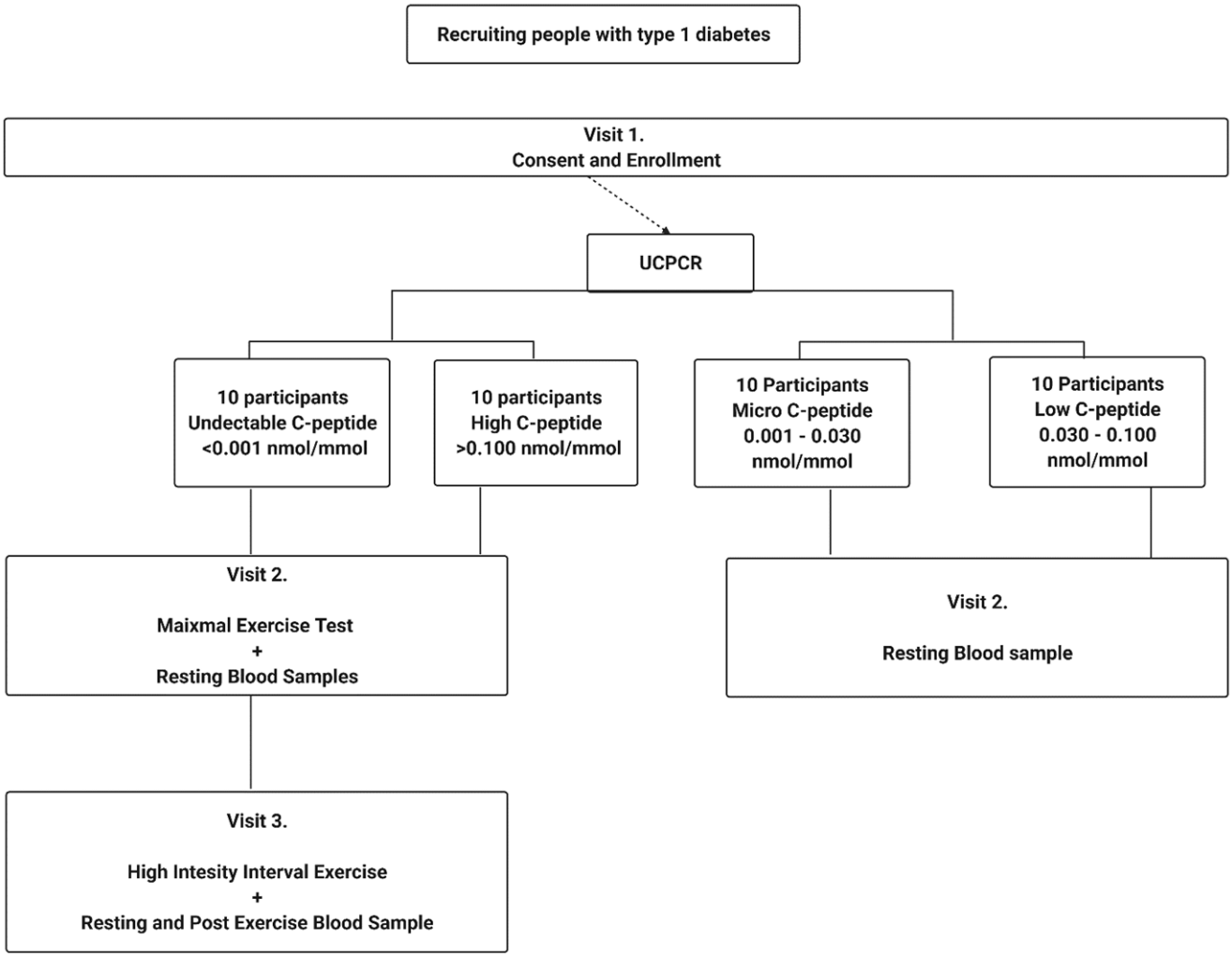
Withdrawal of participants

Participants are free to withdraw from the study at any time, and do not have to provide a reason.

Participant flow

The trial is expected to last approximately 18 months in total (summary figure below). Individuals participating in visit 1 to 3 will be in the study for a maximum of 2 and a half months, whilst participants completing visit 1 to 2 will be in the study for a maximum of 1 month. Data collection will be in the form of biochemical analysis of blood samples, using blood samples to test the function and apoptosis if cell cultures, clinical characteristics, and exercise test results.

Before visits 2 and 3, participants will be asked to fast overnight (>10 hours) and to avoid alcohol consumption and any form of moderate to vigorous exercise for at least 24 hours. For female participants, visit 3 will be scheduled at the early follicular phase of their menstrual cycle or placebo pill phase of the oral contraceptive, to control for oestrogen concentration.



Visit 1. Screening and informed consent

Participant will attend having been sent the participant information sheet prior to the visit. The participant will be allowed as much time as they wish to consider the information and have the opportunity to call the research team, their GP or consult other independent parties to ask any questions before they decide whether they will participate in the study.

During the visit, the Participant Information and Informed Consent will be presented to the participants again, and will detail: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

Participants will undertake a baseline assessment, Physical Activity Readiness Questionnaire for Everyone (PAR-Q+ 2020) and medical questionnaire screening. Medical and pre-test questionnaire includes questions on:

- Ethnicity
- Marital Status
- Education
- Employment
- Medical Conditions
- Smoking Status
- Family health history
- Current medication
- Injuries
- Current Exercise habits
- Blood sampling contradictions
- Bioelectrical impedance contraindications
- Menstrual Status and medication

Baseline assessment will include:

- Height and weight (subsequently BMI)
- Systolic and diastolic blood pressure
- Body composition
- Blood glucose
- Peak flow

Participants with T1D will be given a 2 hour post-prandial urine C-peptide Creatinine ratio (UCPCR) test to complete at home and freepost to Exeter University. The UCPCR will be used to assess residual β-cell function. This test has good sensitivity and specificity for identifying C-peptide thresholds[6,23].

UCPCR will be used to recruit:

- 12 participants with T1D and undetectable C-peptide (<0.001 nmol/mmol)
- 12 participants with T1D and micro C-peptide (0.001 – 0.030 nmol/mmol)
- 12 participants with T1D and low C-peptide (0.030 – 0.100 nmol/mmol)
- 12 participants with T1D and high C-peptide (>0.100 nmol/mmol)

Once 12 individuals have been recruited into each category, individuals who return a UCPCR into a full category will not continue in the study, but will be contacted in the case of dropouts.

Visit 2. Resting blood samples, and CPET

All participants will attend the second visit fasted. Participants with T1D will also have avoided injecting bolus insulin or a corrective insulin dose within the last 2 hours.

A resting blood sample will be taken from all participants. Venous blood samples will be taken by phlebotomy, at the antecubital veins (see venous blood collection procedure below). Capillary blood glucose samples will also be taken, with the visit rearranged if the participants with T1D glucose concentration is outside of 3.9-10 mmol/L.

Participants undertaking the exercise component of the study will also undertake a comprehensive health examination, including resting and exercising ECG as well as a CPET.

ECG

A 12-lead ECG with modified limb lead placement will be used to evaluate participants resting and exercising electrical activity of the heart. If any contraindications are noted (such as ST-segment changes that are greater than or equal to 1 mm, left bundle branch block, ventricular paced rhythm, left ventricular or right ventricular hypertrophy), the visit will be stopped and the participants GP or consultant will be informed by letter of the findings. Participants will only be allowed to continue in the study after they have been cleared by a medical professional.

CPET

The CPET protocol will follow national clinical guidelines[25], performed on a cycle ergometer. Briefly, after 3-min of unloaded cycling, work rate will be increased by ~10-20 $W \cdot min^{-1}$ until the limit of tolerance. The work rate increment will be tailored based on the participant's predicted maximal oxygen uptake[26]. Participants will maintain a cadence of 60-75 $rev \cdot min^{-1}$. Breath-by-breath data will be recorded throughout, using a metabolic cart (Cortex Metalyzer®). Heart rate will be recorded with a Polar heart rate monitor, blood pressure will be monitored with an automated sphygmomanometer, and rating of perceived exertion (RPE) will be recorded using the 6-20 Borg scale[27]. The test will continue until volitional fatigue or the participant has any contraindications to exercise (feeling unwell, systolic blood pressure >260 mm Hg, diastolic blood pressure >115 mm Hg) or their cadence drops ≥10 rev·min-1 below 60 rev·min-1 for five consecutive seconds despite strong verbal

encouragement. To determine whether peak effort was reached, heart rate (HR) criteria (reaching 10 beats·min-1 of age predicted max) and respiratory exchange ratio (>1.1) will be checked after the test.

Participants will remain in the laboratory for roughly 10-20 minutes after the exercise test, to make sure of a full recovery (heart rate and blood pressure ~10% of resting values) and to monitor blood glucose concentration.

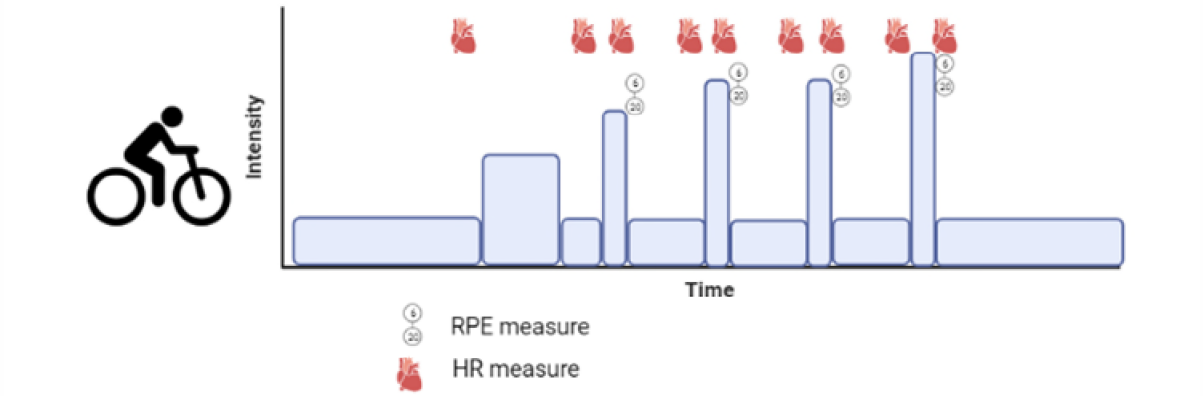
If the participants with T1D capillary blood glucose concentration fall below <5mmol/L anytime throughout the visits they will be given a small carbohydrate snack (15 grams of CHO) to avoid hypoglycaemia. From previous research experience and the wider literature[28,29], blood glucose concentration in individuals with T1D stay largely steady with minimal rises or falls during and after a maximal exercise test.

Visit 3. High Intensity Interval Exercise Bout (HIE) and pre and post-exercise blood samples

Participants will attend fasted on a morning, having avoided exercise for the 48 hours before the trial. Participants with T1D will have maintained their basal insulin rate as normal. Upon arrival, participants will be have a cannula inserted with bloods collected. Blood sample will be taken at rest and as the exercise bout finishes. Capillary blood glucose samples will also be taken, with the visit rearranged if the participants with T1D glucose concentration is outside of 3.9-10 mmol/L. Capillary blood sample will also be used to measure leucocyte count and haematocrit and haemoglobin

Participants will complete a high intensity interval bout on the cycle ergometer as previously described[30]. Briefly, The HII programme will be 21 minutes in duration; 4 min of gentle warm up cycling at 50 W (40–60 rev/min-1), 2 minutes at 80% of maximum work rate (WR) wattage achieved during VO2peak assessment, 2 minutes active recovery at 40% maximum wattage, followed by 2 x 30 seconds at 120% WR max with 2 minutes active recovery between and after, 30 seconds at 150% WR max, before a 5 minute cool down at 50 W (40–60 rev/min-1).. Aim to achieve ≥90% peak heart rate, during the high intensity intervals.

RPE and HR will be recorded throughout. Blood pressure will also be measured in the rest periods. Infographic of the protocol below.



Criteria for terminating the HII exercise:

Participant feeling unwell

Participant experiencing symptoms such as angina, dyspnea, light-headedness, confusion, or signs of poor perfusion.

Rise in blood pressure > 260/115mmHg

Drop in systolic blood pressure >10mmHg from baseline during high intensity interval.

Immediately on completion of the final sprint, whilst the participant is cooling down, blood samples will be drawn from the cannula. A capillary blood sample will be taken to measure blood glucose, leucocyte count and haematocrit and haemoglobin. Upon finishing the cool down, participants will be required to rest for a minimum of 10 minutes, and we will measure heart rate and blood pressure to ensure these have returned to within ≈10% of resting values before participants leave the laboratory.

If the participants with T1D capillary blood glucose concentration fall below <5mmol/L anytime throughout the visits they will be given a small carbohydrate snack to avoid hypoglycaemia. High intensity interval exercise bouts normally raise blood glucose in individuals with T1D[28,31].

Venous Blood Collection Procedure

Venous blood samples will be drawn from a superficial antecubital vein by a trained phlebotomist according to standard laboratory guidelines. During visit 2, venous blood sample will be drawn by phlebotomy with ~60 ml blood samples collected at pre- exercise (rest), while during visit 2, a cannula will be inserted for the drawing of samples with ~60 ml collected pre- and post-exercise.

Visit 2

At rest (pre-exercise) ~60 ml will be collected. Two 5 ml vacutainer tubes containing EDTA for plasma and two 5mL red top vacutainer tubes for serum will be collected, along with four 10 ml heparin tubes for peripheral blood mononuclear cells (PBMC).

Visit 3

Two 5 ml vacutainer tubes containing EDTA for plasma and two 5mL red top vacutainer tubes for serum will be collected, along with four 10 ml heparin tubes for peripheral blood mononuclear cells (PBMC), both at rest (pre-exercise) and post-exercise.

Sample processing

EDTA tubes will be centrifuged immediately at 2,000 x g for 20-min at room temperature to separate plasma. Red top vacutainers will be left at room temperature for ~30-min to allow the blood to clot before being centrifuged. The top layer of serum/plasma will be pipetted off, apportioned into ~0.5 ml aliquots and cryopreserved at 80°C.

Heparinised blood samples will be then poured into a Leukosep tube containing Lymphoprep solution (Axis-Shield) before being centrifuged at 2100 rpm for 12 minutes at room temperature. The cloudy cell layer will be poured into a standard 50ml universal and topped up sterile RPMI medium before this tube is centrifuged at 1600 rpm for 10 minutes to pellet the peripheral blood mononuclear cells (PBMC). After removal of the supernatant, the pellet will be resuspended in residual buffer. A freezing solution, containing 90% foetal calf serum (FCS) and 10% dimethyl sulphoxide (DMSO) will be added to cryovial containing the PBMCs, before being transferred to a "Mr Frosty" controlled freezing container, which is then put directly into a -80 freezer.

Samples will be collected and processed in a COVID-secure manner (full PPE) and stored in Newcastle University's Sport and Exercise Science laboratory for later analysis.

Blood sample analysis

PLASMA ANALYSIS

The plasma concentration of specific cytokines (e.g. IL-6, IL-10, VEGF, SDF-1, IL-1RA, TNF- α) and hormones (e.g. epinephrine) will be measured using enzyme-linked immunosorbent assays (ELISA) in our Sport and Exercise Science laboratory.

A plasma sample will be sent to the Newcastle Clinical Laboratory for quantifying HbA1c.

PBMCs ANALYSIS

Leukocytes populations (e.g. T Cells, T helper cells, T regulative cells, monocytes, B cells, NK cells) and activation will be assessed by flow cytometry at the Newcastle Flow Cytometry Core Facility (NFCCF).

T-cell reactivity in response to pancreatic proteins (GAD65, C-peptide, IA-2) will be measure by ELISpot assay (measuring IFNγ cytokines).

Endothelial Progenitor Cells Stimulation

Purchased human EPCs will be grown to Passage 2 before harvesting. Cells will be separated and cultured in hyperglycaemic (25mmol/L) or normoglycaemic (5mmol/L) environments. Additionally, the cells will be further separated and grown either without any additional supplementation or with sequential concentrations of C-peptide and/or insulin.

Exposing endothelial cells to serum from differing populations in culture has been demonstrated to impact upon the function[32]. Individual's serum samples will be pooled into group serum comparing participants with T1D and undetectable β-cell function, against participants with T1D and high β-cell, and non-diabetes control participants as well as high β-cell function. EPCs will be supplemented with pooled serum from the different groups. Pooled serum (both pre- and post-exercise) allows reduced inter-individual variation in response[33], allowing the focus on whether the effects of C-peptide supplementation are replicated in an *in vitro* model that is closer to physiological conditions. EPCs function (e.g. scratch assay) and apoptosis (by flow cytometry) will be assessed.

Sample storage

Upon initial collection, samples will froze in the -80 freezers in the CRF or Sport centre labs. Once frozen, and the participants study visit finished, sample will be moved to the Newcastle Biobank for long term storage.

Statistical Analysis

EPCs function and apoptosis will be compared between groups using a one-way ANOVA. When measurements are also across time (e.g. scratch assay, with measurements taken at pre and 2, 4, 6 and 12 hours post scratch) a mixed model ANOVA will be used, comparing groups over time. Counts of resting leucocytes, T-cell reactivity, cytokines and hormones will be compared between groups by a one-way ANOVA.

Changes in counts from pre- to post-exercise between groups will be assessed by a two way mixed model ANOVA. Data that are measured over time (e.g. scratch assay) will be analysed by a three way mixed model ANOVA.

All data will be assessed for normality, and attempted to be transformed if it fails, with non-parametric tests used if not. Analysis will be performed in SPSS and Graphpad.

ETHICAL AND SAFETY CONSIDERATIONS

Cardiopulmonary exercise testing

As with any form of exercise, there is a small risk of cardiovascular complications. However, the risk of an adverse cardiovascular event during supervised CPET in individuals asymptomatic of cardiovascular disease is extremely low. Even in patients with cardiac disease, the incidence rate for a major cardiac event is reported to be 1.2 per 10,000 tests [8]. To put this into context, in 2020 the UK incidence rate of death or serious injury in a road traffic accident was 3.6 per 10,000 people [13]. We will minimise the risk further by implementing the following measures:

- We will follow ACSM guidelines on exercise pre-participation health screening to exclude individuals who are at increased risk for adverse exercise-related cardiovascular events. That is, we will exclude all individuals who show any signs/symptoms of cardiovascular or renal disease. As individuals with T1D have a pre-existing metabolic disease (increasing their risk) a pre-exercise ECG screening will need to be successfully completed for them to participate in the study.
- Hypertensive participants will be excluded (>160/90 mmHg)
- Participants will perform a warm-up and the exercise intensity increases gradually (the rate of increase is individually tailored to the person's predicted fitness level)
- The exercise will be stopped immediately if participants show an exaggerated blood pressure response (>250 mmHg systolic or >120 mmHg diastolic), a fall in blood pressure of >20 mmHG from the highest value during the test, or report feeling

unwell or any discomfort that is symptomatic of cardiovascular complications (e.g. chest pain, unreasonable breathlessness).

- A person trained in First Aid will be present in the Sports Centre and an automated external defibrillator (AED) is located in the laboratories if required.
- Participants will require to rest in the laboratory for a minimum of 10 minutes after exercise cessation. We will measure heart rate and blood pressure to ensure these have returned to within ≈10% of resting values before participants leave the laboratory.
- Participants with any pre-existing injury that could be exasperated by indoor cycling will be excluded from the study.

Covid-19 transmission

There is a small risk of COVID-19 exposure/transmission whilst taking part in the research. This risk will be minimised with the following measures:

- *All research team members will strictly adhere to current government guidance on COVID-19 (https://www.gov.uk/coronavirus). Research participants will also be instructed to do so.*
- *Participants/researchers will be instructed not to attend the laboratory if they: i) have any COVID-19 symptoms, ii) have received an official letter advising that they should self-isolate, or iii) if they or anyone in their household has tested positive in the last 14 days. Participants will be contacted 24 hours before study visits to check they meet this criteria.*
- *Researchers will wear full personal protective equipment (PPE) including gloves, goggles, plastic aprons, and face mask.*
- *PPE will be donned/doffed in the same room as the exercise testing and disposed of in an appropriate clinical waste bin. Hands will be thoroughly washed after PPE is removed.*
- *Researchers will sanitise their hands before participants arrive. Participants will also be asked to use hand sanitiser when they arrive (this will be provided).*
- *All study equipment will be cleaned with surface disinfectant before and after each study visit. Non-essential equipment will be removed from the testing area to reduce cleaning requirements.*
- *Respiratory equipment (CPET facemask and flow sensor/turbine) will be sterilised in sterilising liquid (Milton) for one hour after use. All participants will use a sterile facemask and flow sensor/turbine.*
- *Participants will be required to bring and drink from their own water bottle.*

Blood collection and processing

Although venepuncture is routinely performed in the Sport and Exercise Science laboratory, there is a small risk of cross infection or needle stick injury. The person sampling blood will be a trained phlebotomist and will have demonstrated their competence in supervised attempts to an experienced member of staff. Researchers will follow good practice in venepuncture, as outlined in the laboratory Standard Operating Procedure (SOP). The sampling area will be wiped with a pre-injection swab before the sample is taken. The researcher will maintain communication with the participant to make sure they feel comfortable, and ask them not to look at the sample if they have a history of fainting. A member of the research team will observe the participant for at least 10 minutes after the sample has been taken. Sharps will immediately be disposed of into an appropriate sharps bin. Full PPE will be worn by researchers during the blood sampling and processing, including gloves, goggles, plastic aprons, and face mask. Participants reporting any known blood-borne viral infections or those who have previously tested positive for HIV, Hepatitis B or Hepatitis C will be excluded from the study.

Ethical approval

Ethical approval for the participants with type 1 diabetes attending the CRF at the RVI hospital will be applied for through the REC and HRA ethics committees. For the participants without diabetes taking place in the Newcastle University Sports Centre Labs, ethical approval will be applied for through Newcastle University Medical School ethics department.

Definition of End of Study

The study will be finished once all participants have completed data collection. Samples will be analysed during and after study completion.

Safety Reporting

Definition of Serious Adverse Events

An unplanned adverse event leading to hospitalisation, long term disability or death.

Reporting Procedures for Serious Adverse Events

Reported to REC committee within 15 days of becoming known to the study team.

Data Management

Access to data

Only named study team will have access to the participant identifiable information. Any individual with access will be added to the signed delegation log.

Data recording and record keeping

Data will be recorded in a password protected master sheet. Data will be updated after every study visit. Upon completion of the study, the raw data sheet will be locked and any alterations (such as excluding outlier data) saved as a new version.

Protocol Deviations

Any changes in protocol will be reported to sponsor, ethics committee and funder. If protocol deviations are deemed as substantial as per the REC committee, the study will undergo a subsequent ethical review.

ETHICAL AND REGULATORY CONSIDERATIONS

Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

Approvals

Following Sponsor approval the protocol, informed consent form, participant information sheet and any additional study documentation will be submitted to an appropriate Research Ethics Committee (REC), and HRA (where required) and host institutions for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Reporting

The CI shall submit once a progress report to the REC Committee, HRA, host organisation, Sponsor and funder, if required. In addition, an End of Study notification and final report will be submitted to the same parties.

Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

Participant Confidentiality

The study will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

Expenses and Benefits

Participants will receive a £20 voucher for each of visit 2 and 3 they completed, once they have finished in the study. This is to reimburse participants time and encourage retention for the study, and cover any reasonable travel expenses. Participants may benefit from

taking part in this study as they will receive information on their fitness, body composition, residual β-cell function and blood glucose control.

FINANCE AND INSURANCE

Funding

The study will be funded by Diabetes Research and Wellness Foundation (DRWF) research grant to Guy Taylor. The research funding will be administered by the Newcastle University.

Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research.

PUBLICATION POLICY

The trial results will be published and all who meet the criteria for authorship will be listed as authors. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. The funders will have no role in decisions on publication but authors will acknowledge the funding source.

Participants will be informed of the trial results through an information sheet prepared for a lay audience that will be made available via email.

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