An Experimental Series Investigating The Effects Of Hyperinsulinemic Euglycemia On Myocardial Blood Flow Reserve In Healthy Individuals And On Myocardial Perfusion Defect Size Following ST-segment Elevation Myocardial Infarction

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Abstract

Background: Incomplete restoration of myocardial blood flow is reported in up to 30% of ST-segment elevation myocardial infarction (STEMI) despite prompt mechanical revascularisation. Experimental hyperinsulinemic euglycemia (HE) increases myocardial blood flow reserve (MBFR). If fully exploited, this effect may also improve myocardial blood flow to ischemic myocardium. Using insulin-dextrose infusions to induce HE, we conducted four experiments to determine: a) how insulin infusion duration, dose, and presence of insulin resistance affect MBFR response; and b) the effect of an insulin-dextrose infusion given immediately following revascularisation of STEMI on myocardial perfusion.

Methods: MBFR was determined using myocardial contrast echocardiography (MCE). Experiment 1 [Insulin duration]: twelve participants received an insulin-dextrose or saline infusion for 120 minutes. MBFR was measured at four time intervals during infusion. Experiment 2 [Insulin dose]: twenty-two participants received one of three insulin doses (0.5, 1.5, 3.0mU/kg/minute) for 60 minutes. Baseline and 60-minute MBFR's were determined. Experiment 3 [Insulin resistance]: five metabolic syndrome (MetS) and six type-2 diabetes (T2DM) participants received 1.5mU/kg/minute of insulin-dextrose for 60 minutes. Baseline and 60-minute MBFR's were determined. Experiment 4 [STEMI]: following revascularisation for STEMI, twenty patients were randomised to receive either 1.5mU/kg/minute insulin-dextrose infusion for 120 minutes or standard care. MCE was performed at four time intervals to quantify percentage contrast defect length.

Results: Experiment 1: MBFR increased with time through to 120 minutes in the insulin-dextrose group and did not change in controls. Experiment 2: compared to baseline, MBFR increased in the 1.5 (2.42±0.39 to 3.25±0.77, p=0.002), did not change in 0.5, and decreased in 3.0 (2.64±0.25 to 2.16±0.33, p=0.02) mU/kg/minute groups. Experiment 3: compared to baseline, MBFR increase was only borderline significant in MetS and T2DM participants (1.98±0.33 to 2.59±0.45, p=0.04, and 1.67±0.35 to 2.14±0.21, p=0.05). Experiment 4: Baseline percentage contrast defect length was similar in both groups but with insulin decreased with time and was significantly lower than control at 60 minutes (2.8±5.7 vs 13.7±10.6, p=0.02).

Conclusions: Presence of T2DM, insulin infusion duration, and dose are important determinants of the MBFR response to HE. When given immediately following revascularization for STEMI, insulin-dextrose reduces perfusion defect size at one hour. HE may improve MBF following ischemia, but further studies are needed to clarify this.

Keywords: diabetes; euglycemic hyperinsulinemia; insulin; microvascular obstruction; myocardial blood flow; myocardial contrast echocardiography; myocardial infarction.

Introduction

Despite advances in stent technology and timely mechanical revascularisation of epicardial coronary arteries for the contemporary management of ST-segment elevation myocardial infarction (STEMI), mortality remains unchanged over the last decade.[1] Incomplete restoration of myocardial blood flow (MBF) in up to a third of myocardial infarctions despite successful epicardial coronary artery revascularisation may account for this at least in part.[2] This persistent MBF deficit is largely attributed to microvascular obstruction (MVO) and adversely affects myocardial remodelling and prognosis.[3] Therefore, there remains an ongoing need to develop treatments that improve MBF in the setting of acute myocardial infarction.

In addition to its metabolic actions, insulin plays an important role in endothelial function, where it has been shown to upregulate nitric oxide (NO) and promote vasodilatation.[4] Our group has previously shown that experimental hyperinsulinemic euglycemia (HE) increases myocardial blood flow reserve (MBFR) - the ratio between hyperemic and resting myocardial blood flow.[5] MBFR represents the maximum capacity of the coronary circulation to dilate and thus augment flow following an increase in myocardial metabolic demand, such as during ischemia.[6] Since myocardial perfusion is predominantly determined by the coronary microcirculation, we speculate that this endothelial vasodilator activity could further increase MBF following successful coronary revascularisation for STEMI. Previous clinical trials of insulin administration during acute coronary syndromes (ACS) adopted insulin protocols with the intent of metabolic optimisation, thus this effect was not fully exploited.

Abbreviations: ACS = Acute coronary syndrome; HE = Hyperinsulinemic euglycemia; ID = insulin-dextrose; MBF = myocardial blood flow; MBFR = myocardial blood flow reserve; MCE = Myocardial contrast echocardiography; MetS = Metabolic syndrome; NO = Nitric oxide; STEMI = ST-segment elevation myocardial infarction; T2DM = Type-2 diabetes mellitus.

From a vasodilator perspective, we have previously discussed potentially limiting factors with respect to insulin-dosing.[7] For instance, the time taken for HE to exert an effect, and a safe yet potent insulin dose, may significantly alter the coronary microvascular vasodilator response. Furthermore, it is uncertain whether the presence of metabolic syndrome (MetS) and type-2 diabetes mellitus (T2DM) impedes the insulin-mediated vasodilatory effect.

If experimental HE can increase MBF, then factors influencing maximum exploitation of this property require definition prior to clinical application aiming to improve MBF during myocardial infarction. We hypothesise that in the context of pharmacologically induced HE, a minimum insulin-dextrose infusion time, a sufficient insulin infusion dose, and normal insulin sensitivity, may be influential in maximising MBFR response. If these conditions are met, HE may augment myocardial blood flow further following revascularisation for acute myocardial infarction.

Therefore, in order to address these areas of uncertainty and specifically to assess the effect of HE on myocardial perfusion in STEMI, we performed a series of experiments with the following aims. First, to assess the relationship between HE duration and MBFR. Second, to assess the relationship between insulin infusion dose and MBFR. Third, to assess the effect of metabolic syndrome and type-2 diabetes mellitus on the MBFR response to HE. Finally, to adopt the findings of these three experiments to design and conduct a clinically feasible trial protocol to test whether HE increases myocardial perfusion in the setting of STEMI.

Materials and Methods

This study was a set of prospective feasibility experiments conducted in the Sunshine Coast Hospital and Health Service, Queensland, Australia (January 2016 - August 2017). All participants provided written informed consent. Experimental protocols were approved by the local research ethics committee (HREC/15/QPAH/386), and registered with the Australian New Zealand Clinical Trials Registry

(ACTRN12617000845336). Protocol schematics and recruitment flow diagrams are illustrated in *Supplementary Methods*.

Healthy Volunteer Experiments

Participants: Volunteers recruited from local community advertisements participated in three experiments. All participants were without documented coronary artery disease and underwent exercise stress echocardiography to exclude obstructive coronary artery disease or structural heart disease prior to enrollment. Prior to each experiment, participants fasted for eight-hours, and were abstinent from alcohol and caffeine for a minimum of 24 hours. Participants were instructed to take all prescribed regular medications (*Supplementary Methods*).

Experimental protocols:

(See Statistical analysis section for sample size calculation)

Experiment 1: Effect of insulin duration on MBFR.

Twelve healthy participants not taking regular medications were randomly assigned 1:1 to receive a 120-minute intravenous infusion of either 1.5mU/kg/minute of insulin with a titrated infusion of 25% dextrose (insulin-dextrose group), or 0.9% saline only (control group). MBFR assessment using vasodilator adenosine MCE was undertaken at baseline, and then repeated at 30, 60, and 120 minutes after insulin infusion commencing. Adenosine was used because of its short half-life for the purpose of serial MBFR assessment. Vasodilator protocols for MBFR assessment are described in *Supplementary Methods*.

Experiment 2: Effect of insulin dose on MBFR.

Twenty-two participants were randomly assigned to one of three insulin infusion doses: 0.5 (n=7), 1.5 (n=7), and 3.0 (n=8) mU/kg/minute with a titrated infusion of 25% dextrose. The insulin-dextrose infusion was administered for 60 minutes. Participants underwent initial assessment of MBFR without insulin (baseline MBFR), and then again following 60 minutes of insulin-dextrose infusion (ID MBFR). Each MBFR

assessment was performed using vasodilator dipyridamole MCE. Dipyridamole was used in experiments 2 and 3 due to its greater subject tolerability.

Experiment 3: Effect of T2DM on MBFR

Two groups of participants were recruited according to previously diagnosed metabolic syndrome (MetS) (n=5), and type-2 diabetes mellitus (T2DM) (n=6). Metabolic syndrome was defined using the International Diabetes Federation 2005 definition (*Supplementary Methods*).[8] The T2DM group had been previously diagnosed by their usual medical practitioner. Additional comparison was made with data obtained from the 1.5mU/kg/minute insulin dose group (experiment 2, n=7) to represent a 'control' group. All participants received an insulin infusion dose of 1.5mU/kg/minute with a titrated infusion of 25% dextrose. The experimental protocol was otherwise identical to that of Experiment 2.

Hemodynamic parameters:

The rate-pressure product (RPP) was calculated as the product of HR and SBP at each measurement. Previous studies have found that euglycemic hyperinsulinemia increases HR and SBP, which indirectly increases MBF consequent to an increase in cardiac output.[9]

Biochemistry:

Laboratory serum insulin and glucose concentrations were measured at baseline, and then repeated immediately after each MBFR assessment. An immunoenzymatic assay with chemiluminescence detection was used to quantify insulin (Unicel DxI 800 Immunoassay System, Beckman Coulter, Brea, California, USA). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from the formula: fasting insulin (mU/L) x fasting blood glucose (mmol/L) / 22.5.[10] Hemoglobin, renal function, and lipid profile were also measured, as these are all potential confounding variables on MBF.[11-13]

Experiment 4: STEMI

Participants: patients presenting with STEMI for emergent PCI were invited to participate in the study. Exclusion criteria included previous MI, coronary or valvular intervention or surgery, diabetes mellitus on insulin therapy, cardiogenic shock, inability to provide written informed consent, or have a known terminal illness.

Protocol:

After successful coronary revascularisation and transfer to the coronary care unit, all patients received a baseline myocardial perfusion assessment using MCE. Patients were then randomised 1:1 to receive either a 120-minute insulin-dextrose (ID) infusion or guideline-directed care alone (controls). Myocardial perfusion assessment was repeated at 30, 60, and 120 minutes after commencement of ID. After the final MCE study, insulin was discontinued and glucose slowly down-titrated over a 30-minute period in the ID group.

ST-segment elevation resolution:

ECG ST-segment resolution at 90 minutes after reperfusion was used for semi-quantitative analysis of coronary reperfusion, with an ST-segment resolution of >70% defined as complete resolution.[14]

Biochemistry:

In the ID group, laboratory serum insulin and glucose concentrations were measured at baseline, and then repeated at 30, 60, and 120 minutes after initiation of ID.

Insulin-Dextrose infusion:

Insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) and 25% dextrose (Baxter Healthcare, NSW, Australia) were infused via an intravenous cannula inserted into an antecubital vein of the left arm. Venous blood was drawn from an intravenous cannula inserted into a right arm vein. Insulin was commenced at the corresponding study dose after baseline MBFR assessment. Euglycemia, defined as blood glucose ≥

4.0mmol or within 10% of baseline, was maintained using a titrating dose of 25% dextrose. Dextrose infusion was commenced at 4 minutes after insulin commencement at an initial rate of 2mg/kg/min, and adjusted according to blood glucose measured every 5 minutes using a commercial glucometer (VerioIQ, Johnson and Johnson, New Jersey, USA), which has been validated against the criterion glucose-oxidase method.[15]

Myocardial perfusion assessment using myocardial contrast echocardiography (MCE):

Myocardial contrast echocardiography was performed using commercial ultrasound machines (iE33 or EPIQ, Philips Medical Systems, Best, the Netherlands) and Definity contrast agent (Lantheus Medical Imaging, MA, USA) with flash microbubble destruction as previously described.[6] Briefly, real-time images were recorded in the three apical views (4-chamber, 2-chamber, and 3-chamber) with low-power settings at a mechanical index of 0.1. The focus was set at the mitral valve level. Definity was gently rotated throughout the experiment to maintain the contrast agent in suspension, and was initially infused intravenously at 200 mL/h. Thereafter, the rate was set between 150 and 200 mL/h to maximize image quality with minimal attenuation. Once optimized, the ultrasound settings were held constant throughout the experiment. Flash-impulse imaging at a high mechanical index (1.0) was performed to achieve complete myocardial microbubble destruction, after which 10 end-systolic frames were recorded digitally in each apical view.[16] In Experiments 1-3, after the resting images were acquired, vasodilator images were acquired during maximal endothelium-independent hyperemia using adenosine or dipyridamole from clinical pharmacological stress-testing dosing protocols.[17] Continuous ECG monitoring was undertaken, and systolic blood pressure (SBP) and heart rate (HR) was recorded before and after administration of vasodilator agents.

Image analysis:

All MCE studies were analysed blinded to treatment received (MN) a minimum of 2 weeks after experiment completion. Quantitative MCE analysis was performed offline using commercially available

software, QLab version 10.4 (Q-Laboratory, Philips Medical Systems, Eindhoven, Netherlands). Examiners were blinded to participant details and insulin-dextrose infusions received in all experiments. Quantitative assessment of myocardial perfusion was performed for 10 consecutive end-systolic frames after microbubble destruction. Analysis was performed on up to 10 segments of the 17-myocardial-segment model. The basal segments being furthest away from the ultrasound transducer were not included because of contrast attenuation.[6] A region of interest was placed over the entire thickness of the myocardium, and particular care was taken to exclude high-intensity epicardial and endocardial borders by manually moving the region of interest between each frame (Figure 1). Background-subtracted plots of peak myocardial contrast intensity versus pulsing intervals (representing time) were automatically constructed by QLab software and fitted to a monoexponential response curve: $y=A\ (1 - e^{-\beta t})$. From this, the slope of the replenishment curve (β [dB/s], representing myocardial blood velocity) and the peak intensity (A [dB], representing myocardial blood volume) were derived, and the product of A and β yielded myocardial blood flow (MBF). In the healthy volunteer experiments, MBFR was calculated as the ratio of hyperemic to resting MBF for each segment and then calculating the mean. Quantitative MCE was considered feasible if at least five of the 17 segments per patient were analysable.

STEMI perfusion assessment:

From native MCE images, the length of the endocardial border corresponding to the segment of the myocardium with no or poor opacification was measured in the 2-4- and 3-chamber views. The sum of endocardial border length measurements defined the size of the perfusion defect. The following formula was used to assess the relative contrast defect length (CDL%): (total length of residual contrast defect after reperfusion)/(total length of endocardial border) x 100[18] (Figure 4).

Analysis was then repeated to regionalise MBF into infarct-territory MBF and remote MBF by separate analysis of infarct and remote myocardial segments[19]. Ischemic segments were identified as myocardial segments supplied by the infarct-related artery and showing hypokinesia at initial transthoracic

echocardiography conducted on presentation. Remote segments were defined as non-infarct-related artery supplied myocardial segments.

Within all studies, 50 random myocardial segments were reanalyzed blindly for intra- and inter-observer variability at least 2 weeks after initial analysis (JQ). The intraobserver and interobserver variabilities of MBF were 12% and 15%, respectively. The intraobserver and interobserver variabilities of %CDL were 4% and 6%, respectively.

Statistical analysis:

Normality of the data distribution was assessed using a Shapiro-Wilk test. Depending on normality of the data distribution, continuous variables are presented as means and standard deviations or medians and interquartile ranges. Ordinal and dichotomous variables are summarised using proportions or percentages. For comparisons between treatment groups, unpaired student t-tests were used for normally distributed continuous data, Wilcoxon rank-sum tests for non-parametric continuous data, or Fisher's exact tests for binary data. For multiple group comparisons, depending on the distribution of the data, ANOVA or Kruskal-Wallis H tests were used to compare means between dose groups (experiment 2) and diabetic state groups (experiment 3). All analyses were performed using STATA™ version 14.1 and SPSS™ version 25. The level of significance was set at $p < 0.05$.

Healthy volunteer experiments: our group previously showed that euglycemic hyperinsulinemia results in a 22±15% increase in MBFR in healthy individuals.[5] Based on these data and the means and SDs in these individuals, an alpha error level of 0.05, and a power of 80%, we estimated that a minimum of five participants in each experiment group were required to show the same effect, relative to control or between conditions. For the cross-sectional time series, mixed effects linear regression was performed using MBFR as the dependent variable. Within the model, the coefficient for the effect of time on MBFR is expressed as β.

STEMI experiment: there are no published studies exploring serial measurements of myocardial perfusion defect size at such short time intervals immediately following revascularisation so estimating effect size prospectively was speculative. To detect a 15% difference in %CDL and assuming a standard deviation of 10%, nine patients are required in each group for 80% power and an alpha of 0.05. Repeated measures ANOVA was used to assess changes in insulin levels. The groups were coded as zero (0) for the control group and one (1) for the experimental (insulin) group. The data present as a longitudinal time series (panel set) and were analysed as such using a mixed linear model with the patients as the panel variable. The dependent variable was %CDL whilst the independent variables were the experimental group and the observation times (minutes). Validity of the model selection was confirmed by performing a Hausman Test. The regression slopes were recorded as β (g) for the dichotomous group variable and β (t) for the time variable. The same analysis was repeated with global, remote and ischemic MBF as the independent variables.

Results

Tables 1 and 2 summarise experiments 1-3 participant demographics, biochemical and hemodynamic baseline data. In the 1.5mU/kg/minute group, HOMA-IR and fasting insulin levels were significantly higher than the 0.5 and 3.0mU/kg/minute groups but still within the normal range. BMI was higher in the MetS group compared to control (32.9±4.3 vs 25.3±3.2 kg/m^2, p=0.01). Fasting blood glucose was higher in the T2DM compared to the control group (6.2±1.2 vs 5.0±0.2 mmol/l, p=0.04). In the T2DM group, four participants were receiving oral hypoglycemic therapy, and 1 was receiving long-acting insulin. Absolute MBF values are listed in the *Supplementary Tables*.

Experiment 1. Effect of Insulin duration on MBFR

From a baseline of 16.7±11.8 pmol/L, mean serum insulin levels in the insulin-dextrose group were 287±194, 369±138, and 346±158 pmol/L at 30, 60, and 120 minutes after insulin-dextrose commencement,

respectively (p=0.03). In the control group, serum insulin did not change across time points, with a mean overall insulin level 14.6±15.3 pmol/L. Serum glucose did not change significantly from baseline at each measured time interval in both groups.

In the insulin-dextrose group, RPP increased from baseline (7513±1542bpm.mmHg) to 9299±2141, 9392±1762, and 9423±1624bpm.mmHg at 30, 60, and 120 minutes, respectively (p=0.01). RPP did not change in the control group from baseline at the same corresponding time points (p=0.09).

The mean number of analysed myocardial segment pairs per study was 8±1. In the control group, MBFR did not significantly change with time (β=0.00, P=0.80). In the insulin-dextrose group, MBFR increased with time (β=+0.01, P=0.001) (Figure 2A). Regarding specific time points, compared to the MBFR at t=0, MBFR increased from 2.5±0.2 to 3.2±0.7 at 30 minutes (p=0.05), to 3.5±0.6 at 60 minutes (p=0.01), and to 4.1±0.7 at 120 minutes (p=0.004).

Experiment 2. Effect of Insulin infusion dose on MBFR

Insulin levels at 60 minutes of insulin-dextrose infusion were 56±63, 431±132, and 653±354 pmol/L in the 0.5, 1.5, and 3.0mU/kg/minute groups respectively (p=0.002). Across the same respective groups, mean blood glucose at 60 minutes were 4.6±0.75, 5.1±0.73, and 4.7±0.95 mmol/l (p=0.48).

RPP did not change significantly between baseline and following 60 minutes insulin-dextrose infusion in any group. Comparing baseline to 60 minutes, HR did not change in the 0.5 and 1.5, but increased in the 3.0mU/kg/minute group from 60±8 to 69±14 (p=0.04) bpm. Mean SBP fell from 138±16 to 129±26 mmHg in the 0.5mU/kg/minute group (p=0.002).

The mean number of myocardial segment pairs analysed were 7±1. Baseline MBFR did not differ significantly across the three dose groups. In the 1.5mU/kg/min group, baseline MBFR increased from

2.42±0.39 to an ID MBFR of 3.25±0.77 (p=0.002). In the 0.5mU/kg/min group there was no change (2.22±0.40 to 2.07±0.55, p=0.54). However, in the 3.0mU/kg/min group, baseline MBFR decreased from 2.64±0.25 to 2.16±0.33 (p=0.02) (Figure 2B).

Experiment 3. Effect of metabolic syndrome and type-2 diabetes mellitus on MBFR

Insulin levels at 60 minutes of insulin-dextrose infusion were 431±132, 493±462 and 285±167 pmol/L in the control, MetS, and T2DM groups, respectively (p=0.25). Three blood samples taken for measuring insulin levels in the T2DM group had partially hemolysed and therefore not included for analysis. Across the same respective groups, blood glucose levels at 60 minutes were 5.1±0.67, 5.5±0.97, and 5.8±1.44 mmol/l (p=0.54).

SBP, HR, and RPP did not change significantly between baseline and 60 minutes in all three groups.

The mean number of myocardial segment pairs per study analysed were 7±2. Baseline MBFR was significantly lower in the T2DM compared to the control group, (1.67±0.35 vs 2.42±0.39, p=0.007) (Figure 3). Baseline MBFR increased in the control group to an ID MBFR of 3.25±0.77, (p=0.002), but were of borderline significance in the MetS (2.59±0.45, p=0.04), and T2DM groups (2.14±0.21, p=0.05). On comparison of ID MBFR across the 3 groups, the MetS group tended to be lower than the control, and higher than the T2DM groups, but this did not reach statistical significance. The ID MBFR in the T2DM group was lower than that of the control group (p=0.04). It is also noteworthy that the ID MBFR in the T2DM group was not significantly different from the control group baseline MBFR (p=0.15).

Experiment 4. STEMI

The results of experiment 1-3 showed that maximal MBFR response from HE was for a minimum of sixty minutes insulin-dextrose infusion time, adopting the 1.5mU/kg/minute insulin dose, and in patients without diabetes. These findings were used to design the experiment 4 protocol.

Twenty-one patients were recruited into the study. Ten received ID, and eleven received guidelines directed therapy (controls) only. Of these, one patient (control) was excluded because of corrupted MCE data. The remaining twenty patients were included in the analysis.

Patient characteristics:

The mean age of the study population was 66±13 years and predominantly male sex (74%). Clinical characteristics are summarised in Table 3. Seventy percent of STEMI's were anterior. There were two patients with 'diet-controlled diabetes' in the control group. All other clinical variables were similar between both study groups.

Insulin-dextrose infusions:

Glucose and insulin levels in the ID group are presented in *Supplementary Tables*. ID infusions were commenced 65±16 minutes after the point of angiographic reperfusion. There were no incidences of hypoglycemia throughout the study.

Myocardial perfusion:

Percentage contrast defect length

Seven patients in the control group and nine in the ID group had contrast defects identified during myocardial perfusion assessment. Immediately following PCI and prior to ID infusion, the baseline %CDL was 12.9±9.5% in the ID group, and 12.7±12.4% in the control group (p=0.96). Univariate regression showed that %CDL changes significantly across time in the insulin group, but not in the control group (p=0.01 vs 0.61) (Figure 5). Furthermore in the multivariable model, %CDL was independently negatively associated with treatment group (β (g) = (-)5.84, p=0.01) and time (β (t) =(-)0.003, p=0.03). On comparison of mean %CDL between ID and control groups at corresponding time points, %CDL was significantly lower

in the ID group at 60 minutes (2.8±5.7 vs 13.7±10.6, p=0.02), but both treatment groups appear to converge at 120 minutes (p=0.42).

Regional quantitative MBF assessment

On regional MBF analysis, control group MBF significantly decreased with time in the infarct-territory segments (β (t) = (-)0.02, p=0.03), but did not change in the remote segments (p=0.2). ID group MBF did not change with time in both infarct-territory and remote segments. Absolute MBF values are listed in the *Supplementary Tables*.

Discussion

This experimental series determined under euglycemic hyperinsulinemic conditions shows that the presence of T2DM, insulin infusion duration and insulin dose are important determinants of the MBFR response to hyperinsulinemic euglycemia. Additionally, when given immediately following revascularization for STEMI and insulin-dextrose infusion reduces perfusion defect size at one hour. Although experimental studies have shown that both hypo- and hyper-glycemia results in reduced myocardial blood flow, this series of experiments reemphasises the hypothesis that insulin administration during myocardial ischemia acts directly by augmenting coronary microvascular function [20].

Insulin has been shown to act directly on the vascular endothelium in-vitro by stimulating release of L-arginine and subsequently NO.[4] The consistent increase in MBFR as a result of euglycemic hyperinsulinemic conditions observed in this dataset is in agreement with previous studies and reemphasises the independent vasodilatory effects of insulin on the microcirculation.[5] However, there have been no previous studies evaluating the effect of insulin-dextrose infusion duration on coronary microvascular blood flow. Our first experiment showed that the peak effect of insulin-dextrose on the coronary microcirculation is not immediate, but progressively increases MBFR in a time-dependent manner. If

insulin-dextrose infusions can increase myocardial perfusion during ischemia, commencing treatment early is therefore critical to maximise efficacy.

Our second experiment investigated the effect of three different insulin infusion doses on MBFR during HE. Only one previous study reported that absolute hyperemic MBF increased in a stepwise manner at higher insulin doses.[21] Our results suggest that too low an insulin infusion dose has no significant effect on the coronary microvasculature. However, our finding that the highest insulin dose resulted in a reduction in MBFR contradicts that previous study. One possible reason for the difference is that in the previous study the cohort were all young and male; both factors of which have been shown to affect coronary microvascular function.[22, 23] Furthermore, insulin signalling has been implicated in the release of the potent vasoconstrictor endothelin-1 (ET-1).[24] The vasodilatory effect of insulin-dextrose on MBFR may therefore display both a lower and upper limit 'therapeutic window' dependent on insulin levels.

Our third experiment determined whether T2DM and MetS attenuated the vasodilatory effects of HE on MBFR. Insulin resistance has been shown to be associated with microvascular dysfunction and our data are consistent with these findings, with the T2DM participants displaying a baseline mean MBFR <2.0 (indicating coronary microvascular dysfunction). During HE, although we noted an increase in flow with insulin-dextrose in the T2DM participants, their MBFR response was diminished when compared to controls receiving the same insulin infusion dose. Moreover, although individuals with MetS demonstrated an increase in MBFR in response to insulin-dextrose, the large confidence intervals reflect the heterogeneous response of this group. We speculate that the spectrum of MetS through to T2DM may have a graded deleterious effect on the vasodilatory effects of insulin.

The effect of an insulin-dextrose infusion on myocardial perfusion during the immediate period following revascularisation in STEMI has never been reported. In our study, where we assessed myocardial perfusion in the immediate period following coronary reperfusion, the control group displayed a reduction in MBF in

the ischemic territories from baseline assessment onwards. This phasic decline in MBF is in keeping with experimentally demonstrated transient post-ischemic hyperemia.[25] Furthermore, our results also show that insulin-dextrose infusion administered following successful revascularisation significantly reduces perfusion defect size compared to control by 60 minutes infusion time. Experimental studies have shown that HE predominantly increases peak MBF during pharmacological hyperemia, rather than resting.[21] This implies that its effects are additive under such conditions by augmenting the endothelial response. In a clinical situation, this corresponds to the reactive hyperemia, where it has been shown that coronary vasodilatation is both submaximal and further coronary flow reserve recruitable in the presence of myocardial ischemia.[26] It can therefore be inferred that the effects of insulin on the endothelium are most potent during the transient period following revascularisation.

The difference in perfusion between insulin-dextrose and control groups was lost at 120 minutes infusion time. Due to the paucity of studies investigating post reperfusion MBF, our explanation for this is speculative. If ischemia-induced reactive hyperemia is a transient phenomenon, then this may be a limiting factor for the vasodilatory effect of insulin, which acts as a pharmacological scaffold to the microvasculature thereby prolonging hyperemia. Furthermore, external compressive forces may overcome the protective effects of insulin. Recent cardiac MRI studies exploring the temporal changes in myocardial tissue following STEMI have shown that myocardial oedema and hemorrhage are common after reperfusion and evident within the first 120 minutes.[27] The resultant compressive forces generated may eventually negate the vasodilatory effects of insulin.

Study limitations

Our study cohort was small, and as such, quantitative conclusions must be interpreted with caution. Nonetheless, using available resources, we were able to conduct multiple experiments and identify three important determinants of the MBFR response to insulin, and generate the hypothesis that insulin-

dextrose may improve myocardial perfusion in the immediate period following myocardial revascularisation in STEMI.

It is possible that commencing insulin-dextrose immediately on STEMI presentation may have provided a higher dose effect at the time of revascularisation. However, we did not adopt this trial design for two reasons: firstly, the need for repeated venous glucose sampling during PCI makes management of an insulin-dextrose infusion impractical. Second, from a vasodilator perspective, there is a theoretical risk of worsening myocardial ischemia when given during untreated STEMI by diverting myocardial blood flow to remote myocardial territories.

In summary, during HE, insulin infusion duration, dose, and presence of T2DM, are important factors that determine its vasodilator effects on MBFR. With consideration of these factors, this pilot study showed that insulin-dextrose infusion administered during the immediate reperfusion period following revascularisation for STEMI may result in a reduction of perfusion defect size. However, our study cohort was small and limits generalisability, and as such any quantitative conclusions must be interpreted with caution. Hence it may yet be premature to disregard this inexpensive and readily available therapy as potential adjunctive reperfusion therapy in the management of acute myocardial infarction. Studies are now needed to confirm these changes in the coronary microcirculation after the acute presentation by perfusion assessment and left ventricular remodelling prior to conducting clinical trials.

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Figure 1

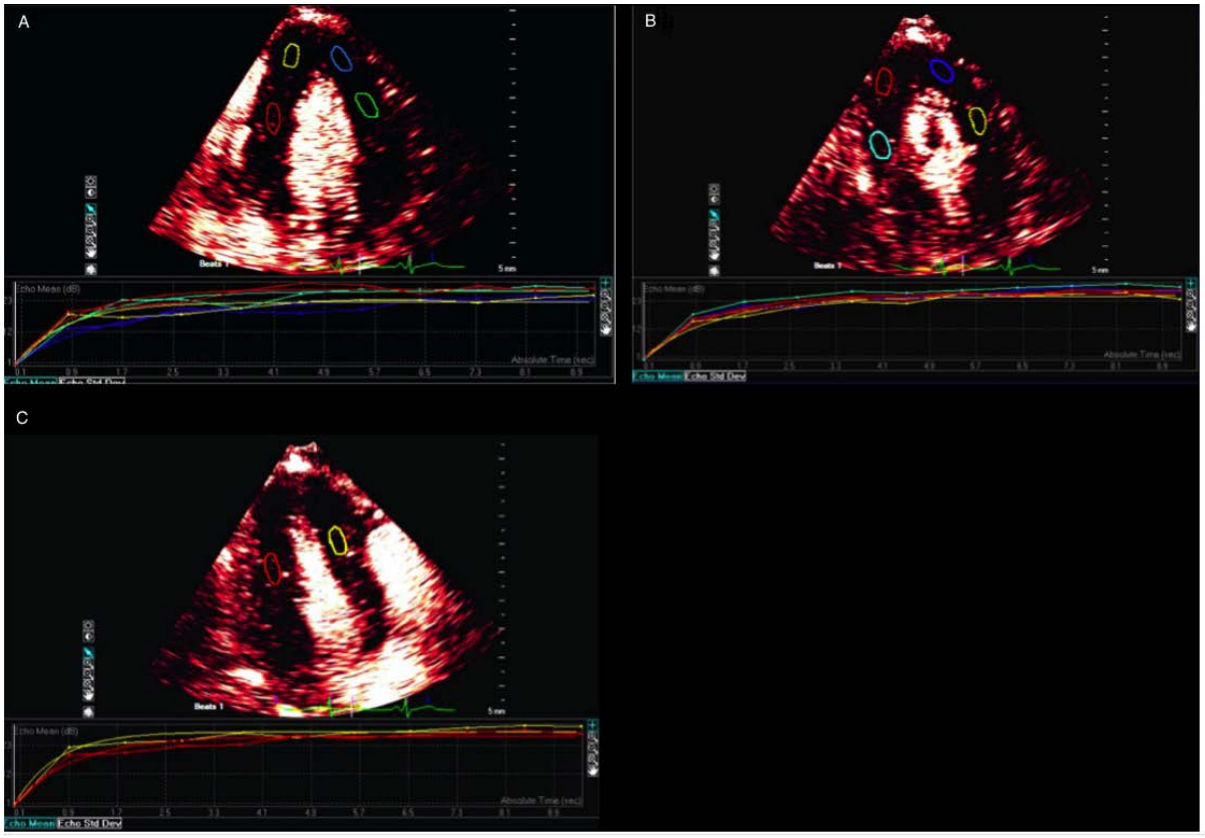

Figure 1: Method used for quantitative analysis of myocardial segments: Apical 4 chamber (A), (Apical 2 chamber (B), Apical 3 chamber (C). Coloured software-constructed replenishment curves below each apical view correspond to each region of interest manually drawn.

Figure 2

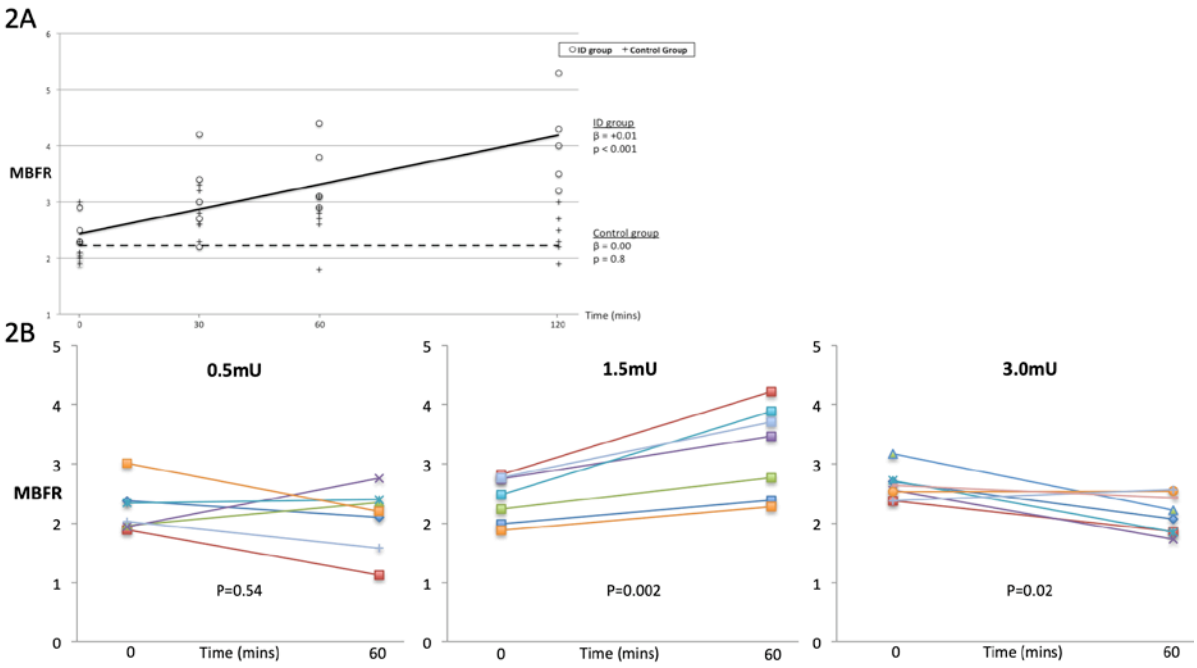

Figure 2A: Effect of insulin duration on myocardial blood flow reserve (MBFR) (experiment 1). Graph showing MBFR at different time points in duration study for insulin-dextrose (ID) and control groups. β and p values were derived from a mixed linear-regression model.

Figure 2B: Effect of insulin dose on myocardial blood flow reserve (MBFR) (experiment 2). Series of graphs each representing the three different insulin dose groups (0.5, 1.5, and 3.0mU/kg/minute) with MBFR at baseline and then following 60 minutes of ID infusion for each participant. P values were derived from comparison of MBFR between 0 and 60 minutes.

Figure 3

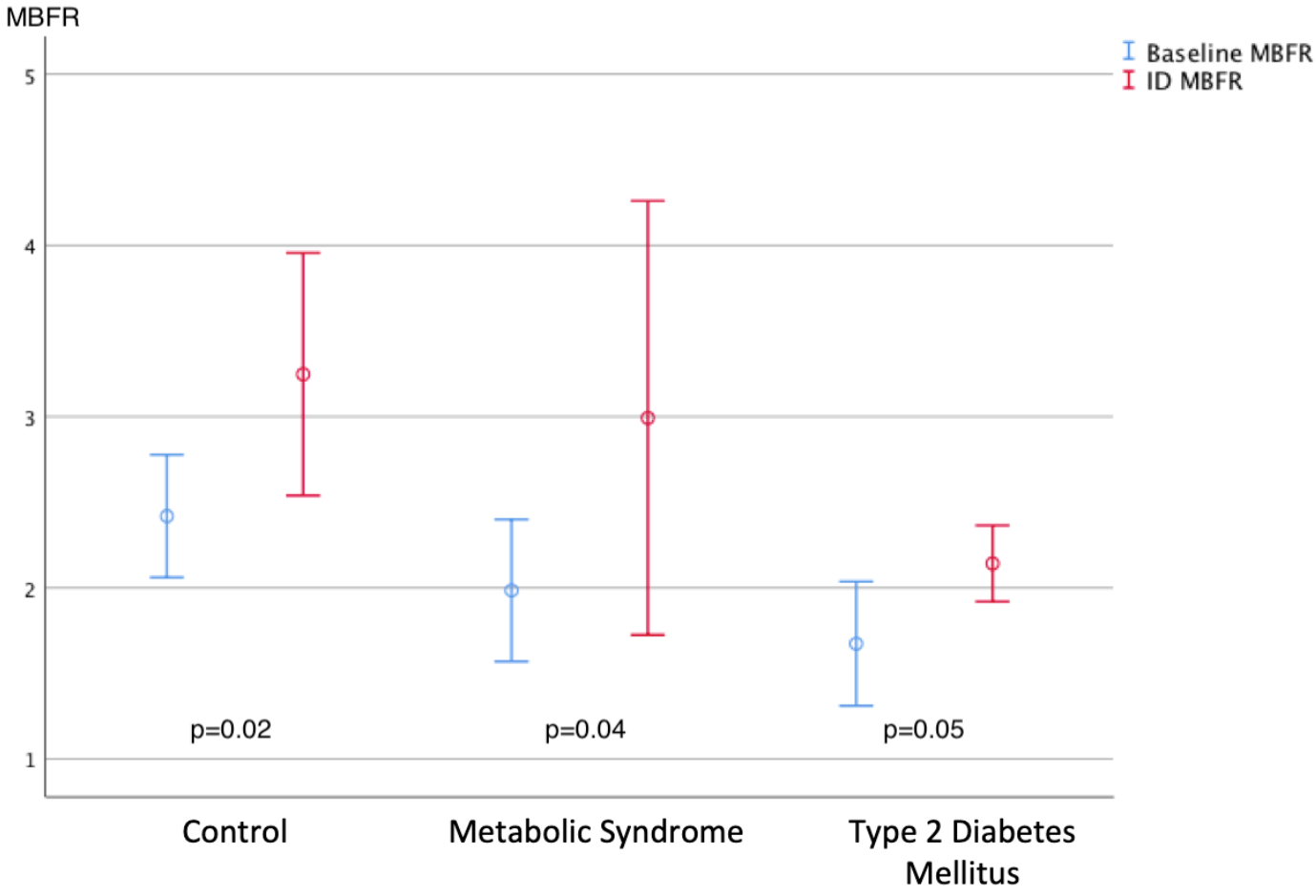

Figure 3: The effect of type-2 diabetes and metabolic syndrome on myocardial blood flow reserve (MBFR) (experiment 3). Mean and error bars showing baseline MBFR and following 60 minutes of 1.5mU/kg/min insulin-dextrose (ID MBFR) in control, metabolic syndrome, and type-2 diabetes groups. P-values are derived from comparison between baseline MBFR and ID MBFR.

Figure 4

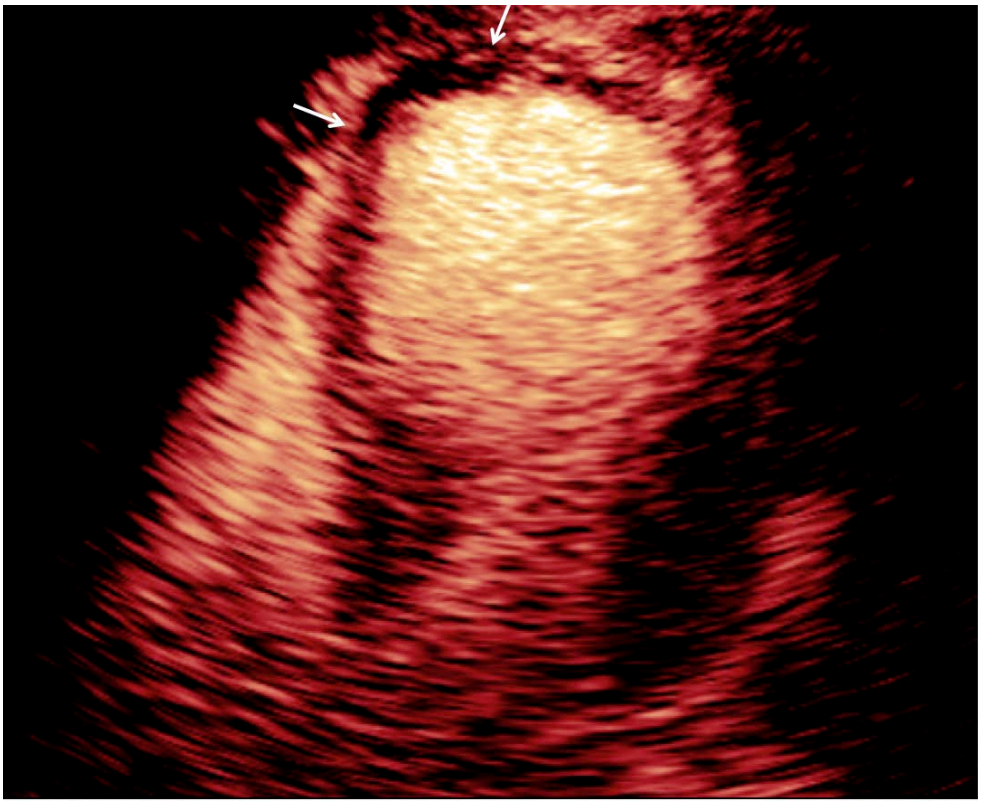

Figure 4: Example of myocardial contrast echo apical 4-chamber view in patient with anterior STEMI and demonstrable contrast defect located at the apex. Contrast defect borders are highlighted by the white arrows.

Figure 5

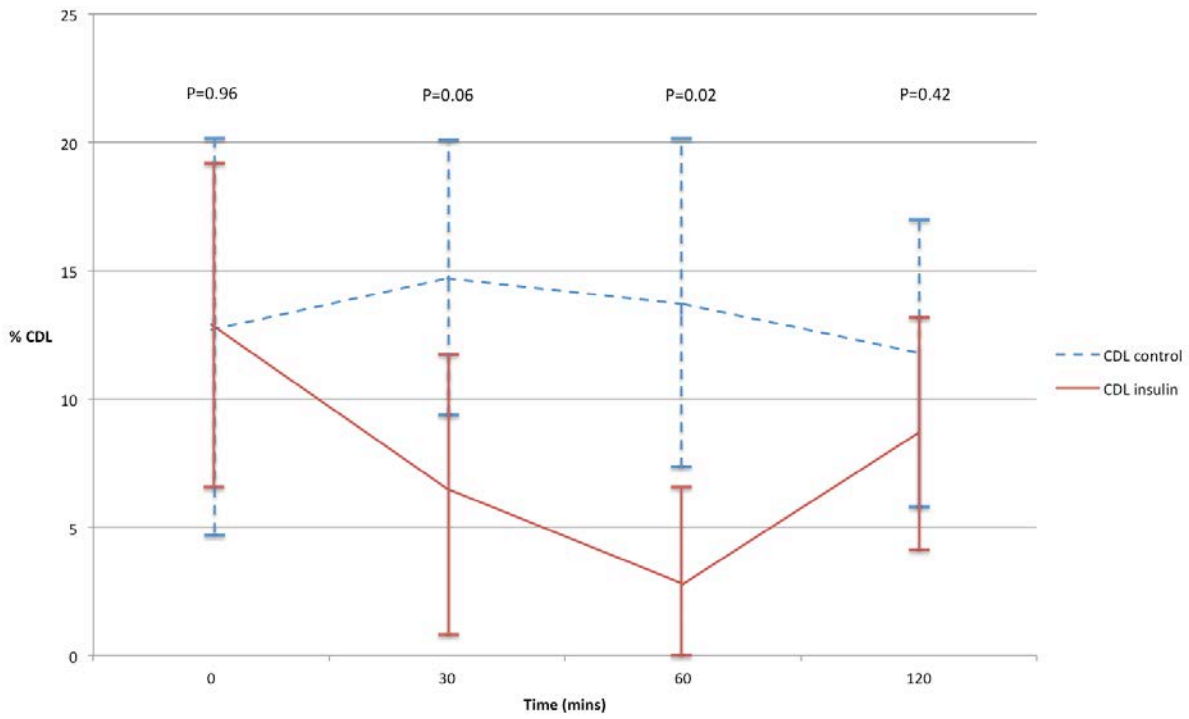

Figure 5: Graph showing mean and 95% confidence intervals of % contrast defect length (%CDL) at pre-specified time-points after baseline MCE study in control (blue) and insulin-dextrose (red) patients. P-values represent comparison between treatment groups using a two-tailed t-test.

Table 1: Effect of insulin duration (experiment 1). Demographic, biochemical, and hemodynamic baseline data of participants in the control and insulin-dextrose (ID) groups

Variable	Control (n=6)	ID (n=6)	P-value
Age (years)	35.5 (6.0)	35.2 (11.2)	0.75
Men (%)	6 (100)	5 (83)	0.34
Body mass index (kg/m^2)	25.7 (2.7)	24.5 (2.3)	0.68
Waist (cm)	89.7 (4.1)	84.7 (7.2)	0.15
Hemoglobin (g/L)	149.0 (8.0)	139.1 (12.5)	0.13
Fasting blood glucose (mmol/L)	4.8 (0.5)	5.1 (0.4)	0.37
Systolic BP (mmHg)	118.7 (7.2)	120.5 (6.8)	0.52
Heart rate (bpm)	58.3 (9.0)	59.5 (11.5)	0.94
Rate Pressure Product (bpm.mmHg)	6955 (1367)	7191 (1589)	0.87
Baseline myocardial blood flow reserve	2.2 (0.4)	2.5 (0.2)	0.09
Insulin (pmol/L)	14.6 (15.3)	16.7 (11.8)	0.40

Values are means (SD) unless otherwise stated

Table 2: Participant characteristics grouped according to insulin infusion dose (experiment 2) and in control subjects or subjects with MetS or type 2 diabetes (experiment 3).

Variable	Experiment 2: Insulin dose (mU/kg/min)				Experiment 3: MetS or T2DM		
	0.5	1.5	3.0		Control	MetS	T2DM
N	7	7	8		7	5	6
Age (yrs)	69.3 (7.6)	61.0 (7.3)	67.9 (6.1)		61.0 (7.3)	65 (7.4)	62 (6.8)
Male (%)	4 (57)	4 (57)	4 (50)		4 (57)	2 (40)	3 (50)
Body mass index (kg/m^2)	24.2 (3.8)	25.3 (3.2)	23.2 (1.9)		25.3 (3.2)	32.9 (4.3)*	30.8 (4.7)
Waist circumference (cm)	85.3 (9.2)	94 (12.3)	85.7 (6.2)		94 (12.3)	107 (5.5)	106 (15.2)
Hemoglobin (g/L)	137.8 (10.2)	143 (10.2)	141.4 (10.1)		143 (10.2)	135 (11.1)	142 (20.1)
Total cholesterol (mmol/l)	5.98 (1.21)	5.77 (1.50)	5.64 (0.79)		5.77 (1.50)	5.58 (0.75)	4.76 (1.03)
Triglyceride (mmol/l)	0.81(0.44)	1.18 (0.95)	0.76(0.23)		1.18 (0.95)	1.86 (0.72)	1.52(0.71)
LDL (mmol/l)	3.93 (1.00)	3.63 (1.09)	3.60 (0.71)		3.63 (1.09)	3.44 (0.85)	2.88 (1.07)
HDL (mmol/l)	1.69 (0.26)	1.59 (0.51)	1.70 (0.43)		1.59 (0.51)	1.29 (0.39)	1.19 (0.27)
Aspirin use (Y) (%)	1 (14.3)	1 (14.3)	0 (0.0)		1 (14.3)	1 (20)	2 (33)
Statin use (Y) (%)	2 (28.6)	1 (14.3)	1 (12.5)		1 (14.3)	2 (40)	2 (33)
ARB/ACEI use (Y) (%)	0 (0.0)	1 (14.3)	0 (0.0)		1 (14.3)	3 (50)	2 (33)
HOMA-IR	0.36 (0.19)	0.68 (0.36)*	0.32 (0.14)		0.68 (0.36)	1.33 (0.54)	1.34 (1.27)
Fasting Insulin (pmol/L)	11.9 (5.49)	21.1 (11.0)*	9.93 (4.17)		21.1 (11.04)	36.8 (12.9)	31.1 (26.7)
Fasting Glucose (mmol/l)	4.69 (0.32)	4.96(0.18)	4.98(0.22)		4.96 (0.18)	5.56 (0.61)	6.2 (1.23)*
Systolic BP (mmHg)	138 (16)	137 (11)	137 (19)		137 (11)	149 (15)	135 (24)
Heart Rate (bpm)	62 (7.8)	59(10.6)	60 (8)		59(10.6)	62 (11)	66 (11)
Rate-pressure product (bpm.mmHg)	8568 (1995)	7988 (2023)	8385 (1817)		7988 (2023)	9343 (2634)	8871 (1945)
Baseline myocardial blood flow reserve	2.22 (0.40)	2.42 (0.39)	2.64 (0.25)		2.42 (0.39)*	1.98 (0.33)	1.67 (0.35)

MetS: metabolic syndrome; T2DM: type-2 diabetes; HOMA-IR: Homeostatic model assessment of insulin resistance; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ARB: angiotensin receptor blocker; ACEI: angiotensin converting enzyme inhibitor.
Values are mean(SD) unless otherwise stated.
Control and 1.5mU/kg/min dose group are an identical cohort and re-tabulated for comparative purposes only.
ANOVA between groups *$p < 0.05$

Table 3: Experiment 4 baseline patient characteristics grouped according to treatment received.

Characteristic	Insulin group (n=10)	Control group (n=10)
Age (yrs)	67.5±13.0	65.6±14.5
BMI (kg/m^2)	28.2±3.4	27.2±6.4
Male sex (%)	8 (80)	6 (60)
Hemoglobin (g/L)	135±13	140±17
Total cholesterol (mmol/L)	5.0±1.1	5.5±1.1
LDL (mmol/L)	3.15±1.09	3.73±0.98
Triglycerides [IQR] (mmol/L)	1.25 [1.00, 2.10]	1.40 [1.00, 1.50]
Left ventricular ejection fraction (%)	48±11	47±12
Wall motion score index	2.3±0.4	2.5±0.5
Troponin I [IQR] (ng/L)	35.4 [6.0, 87.0]	24.6 [0.7, 80.0]
Symptom to revascularisation time [IQR] (minutes)	226 [171, 407]	220 [121, 391]
Reperfusion to experiment commencement (minutes)	41±10	32±11
ST-segment resolution (%)	5(50)	5(50)
eGFR (ml/min)	78±9	74±12
Positive history (%) of:		
Hypertension	1(10)	5(50)
Diabetes mellitus	0(0)	2(20)
Hypercholesterolemia	3(30)	1(10)
Aspirin use	1(10)	0(0)
ACEI or ARB use	1(10)	5(50)
Statin use	2(20)	1(10)
Beta blockers use	0(0)	1(10)
Current or ex-smoker	4(40)	5(50)

$p > 0.05$ for all comparisons between treatment groups

BMI Body mass index, LDL Low density lipoprotein, eGFR estimated glomerular filtration rate, ARB Angiotensin receptor blocker, ACEI Angiotensin converting enzyme inhibitor