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Title Page

Insulin-induced hypoglycemia and the detection of myocardial injury using an ultrasensitive troponin assay

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Hypoglycemia is associated with increased cardiovascular and all-cause mortality in both subjects with and without coronary artery disease (CAD) [1]. Our group recently demonstrated that insulin-induced hypoglycemia (IIH) is associated with a 14% reduction of myocardial blood flow reserve (MBFR) compared to baseline, in both healthy subjects and those with type 1 diabetes mellitus (T1DM) [2]. Cardiac troponin is a sensitive marker of myocardial injury and higher levels have been linked to adverse outcomes in patients with ACS [3]. A new ultrasensitive cardiac troponin I (us-cTnI) has been developed which has a limit of detection at least one order of magnitude lower below that of than current commercial assays. In comparison with current generation troponin assays, us-cTnI has been shown to be more sensitive in the detection of myocardial ischemia. Furthermore, low but elevated levels of us-cTnI in patients presenting with suspected ACS, have been linked to adverse cardiovascular outcomes and death [4]. This study investigated whether 60 minutes duration of symptomatic IIH and reduction in MBFR, was associated with myocardial injury as determined by a change in serum levels of us-cTnI measured immediately following hypoglycemia, in healthy and diabetic subjects without obstructive CAD.

Twenty-three subjects' blood samples were available for analysis from the original study published in 2011 [2]. None were active smokers or have a history of hypertension, CAD or lipid disorder with a normal exercise stress echocardiogram. Insulin sliding scale was commenced in the T1DM group to maintain a glucose level close to 90 mg/dl (5.0 mmol/L). All subjects underwent assessment of MBFR by myocardial contrast echocardiography (MCE) with an insulin clamp at 3 stages: at baseline, during hyperinsulinemic euglycemia (HE) and during hyperinsulinemic hypoglycemia (HH). During HE, insulin and dextrose infusion were used to maintain a glucose level close to 90 mg/dl (5.0 mmol/L) for 60 minutes after an initial 30 minute stabilisation period. Over a 30 minute period, the dextrose infusion rate was reduced to achieve hyperinsulinemic hypoglycemia

(HH) with a target glucose level of 50 mg/dl (2.8 mmol/L) for duration of 60 minutes. During each stage, all volunteers underwent measurement of MBF in 2 states: at rest and after dipyridamole-induced stress. All volunteers provided written informed consent.

Us-cTnI was performed on baseline bloods, at the end of 90 minutes ($t=90$) of HE (30 minutes stabilisation period and then 60 minutes of euglycemia) and at the end of 90 minutes ($t=180$) of HH (30 minutes of stabilisation period and then 60 minutes of hypoglycemia). Us-cTnI was measured using the ultrasensitive Singulex Erenna System (Singulex Inc., Berkeley, CA, USA) which has a limit of detection of 0.2 pg/ml, inter-assay coefficient of variation of 10% at 0.91 pg/ml and 99th percentile value of 9 pg/ml. Blood samples were centrifuged and serum stored frozen at -80°C for later analysis for us-cTnI. Samples were thawed, mixed, and centrifuged before analysis and according to the manufacturer's instructions. Long-term stability of us-cTnI has been demonstrated [5].

Quantitative MCE analysis was performed with commercially available software, Q Lab version 7. MBFR was calculated by the ratio of peak MBF to resting MBF. Further information regarding calculation of MBFR is as previously described [2].

Baseline characteristics of all subjects (16 healthy subjects and 7 T1DM) are described in Table 1. The mean age was 33.3 ± 8.7 and 15(65%) were male. No significant differences were found between the healthy versus T1DM group except a significantly lower fasting plasma glucose in the healthy group ($p < 0.0001$).

The mean MBFR with standard deviation and ranges at baseline ($t=0$), HE ($t=90$) and HH ($t=180$) are as shown in table 2. Mean MBFR for all subjects was 2.48 ± 0.33 at baseline, increasing by 18% during HE to 2.92 ± 0.59 ($p=0.003$), and decreasing to 2.20 ± 0.26 during HH ($p < 0.001$) - a fall of 11.3% and 24.7%, respectively. Subgroup analysis revealed a significant rise in MBFR in the healthy group between baseline and HE (2.55 to 3.11, rise of

22%, $p=0.001$). MBFR fell significantly in the healthy group between HE and HH (3.11 to 2.22, fall of 28.6%, $p<0.001$) and between baseline and HH (2.55 to 2.22, fall of 12.9%, $p=0.001$). No significant differences were observed in the T1DM group comparing baseline versus HE, baseline versus HH and HE versus HH.

Mean us-cTnI with standard deviation and range at baseline ($t=0$), HE ($t=90$) and HH ($t=180$) are as shown in table 2. All values were below the 99th percentile. There were no significant changes in us-cTnI level between time points, for all subjects or when separated into healthy only and T1DM subjects.

The results of this study suggest that immediately following 60 minutes duration of symptomatic hypoglycemia, there was no evidence of myocardial injury as determined by measurement of us-cTnI levels, despite a significant reduction in MBFR. There is increasing amount of evidence linking hypoglycaemia and increased cardiovascular mortality. Multiple theories have been suggested including activation of autonomic system leading to increased myocardial contractility and cardiac stress along with the pro-arrhythmic, prothrombotic and proinflammatory nature of hypoglycaemia [6-8]. The introduction of more sensitive troponin assays permits detection of low levels of myocardial injury. For example, Sabatine et al showed a quantifiable rise of us-cTnI degree of which correlated with amount of ischemia on perfusion imaging after exercise stress testing, while other studies using older troponin assays have failed to show a rise in cardiac biomarker following exercise induced myocardial ischemia [4, 9]. There are several limitations to this study. Firstly, only a small number of patients were recruited. Secondly, it is possible that a rise in us-cTnI levels may have occurred several hours after hypoglycaemia which our sampling may have missed. Thirdly, this study was carried out in a carefully controlled physiological setting with highly specific groups of patients. This should be considered when translating our findings into the dynamic context of acute coronary syndrome which also often involves patients with additional co-

morbidities such as renal dysfunction [10]. Lastly, hypoglycemia in the context of myocardial ischemia may be required to produce a rise in cTnI whereas our subjects had no evidence of ischemia on functional imaging.

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Table 1.

Patient demographics

	Healthy (N=16)	Diabetic (N=7)
Age (years)	33 $\pm$ 8.9	34 $\pm$ 9.6
Men, n (%)	9 (56)	6 (86)
BMI (Kg/m ²)	25.2 $\pm$ 2.7	25.4 $\pm$ 2.8
SBP (mmHg)	115 $\pm$ 11.1	121 $\pm$ 9.8
DBP (mmHg)	72.8 $\pm$ 7.2	78 $\pm$ 8.1
Heart rate (bpm)	81.5 $\pm$ 16.8	74.6 $\pm$ 10.5
Fasting glucose (mmol/L)	4.9 $\pm$ 0.3	8.1 $\pm$ 2.3
Duration of diabetes mellitus (years)		16.1 $\pm$ 9.8
Glycosylated haemoglobin (HbA1c)		8.4 $\pm$ 1.3
Albumin creatinine ratio (mg/mmol)		3.1 $\pm$ 4.8
TC (mmol/L)	4.5 $\pm$ 0.6	4.6 $\pm$ 0.9
LDL-C (mmol/L)	2.2 $\pm$ 0.9	2.4 $\pm$ 0.9

HDL-C (mmol/L)	1.5 ± 0.3	1.8 ± 0.3
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TG (mmol/L)	1.3 ± 1.3	0.9 ± 0.3
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Data is presented as mean ± standard deviation.

BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TC, Total Cholesterol; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; TG, Triglycerides.

Table 2.

Myocardial blood flow reserve (MBFR) and ultrasensitive cardiac troponin (us-cTnI, pg/ml) at baseline, t = 0 min, during hyperinsulinemic euglycemia (HE), t = 90 min and during hyperinsulinemic hypoglycemia (HH), t = 180 min.

	All (N=23)		Diabetic (N=7)		Healthy (N=16)	
Stage	Mean($\pm$ SD)	Range	Mean($\pm$ SD)	Range	Mean($\pm$ SD)	Range
Baseline						
MBFR	2.48(0.33)	(1.73-3.20)	2.32(0.35)	(1.73-2.93)	2.55(0.31)	(2.10-3.20)
us-cTnI	1.82(1.90)	(0.20-7.18)	2.08(2.32)	(0.20-6.96)	1.71(1.76)	(0.45-7.18)
HE						
MBFR	2.92(0.59) [†]	(1.50-4.23)	2.49(0.61)	(1.50-3.33)	3.11(0.50) [‡]	(2.31-4.23)
us-cTnI	1.71(1.70)	(0.16-6.06)	2.07(2.16)	(0.25-6.04)	1.55(1.52)	(0.16-6.06)
HH						
MBFR	2.20(0.26) [‡]	(1.36-2.68)	2.17(0.43)	(1.36-2.68)	2.22(0.16) [‡]	(1.97-2.48)
us-cTnI	1.83(1.75)	(0.40-7.65)	1.91(1.96)	(0.40-5.99)	1.80(1.72)	(0.64-7.65)

Data is presented as mean ($\pm$ standard deviation) and range. For a significant difference between previous stage: * = $p < 0.05$, [†] = $p < 0.01$, [‡] = $p \leq 0.001$.