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Type 2 diabetes mellitus and myocardial ischemic preconditioning in symptomatic coronary artery disease patients

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Abstract

Background: The influence of diabetes mellitus on myocardial ischemic preconditioning is not clearly defined. Experimental studies are conflicting and human studies are scarce and inconclusive.

Objectives: Identify whether diabetes mellitus intervenes on ischemic preconditioning in symptomatic coronary artery disease patients.

Methods: Symptomatic multivessel coronary artery disease patients with preserved systolic ventricular function and a positive exercise test underwent two sequential exercise tests to demonstrate ischemic preconditioning. Ischemic parameters were compared among patients with and without type 2 diabetes mellitus. Ischemic preconditioning was considered present when the time to 1.0 mm ST deviation and rate pressure-product were greater in the second of 2 exercise tests. Sequential exercise tests were analyzed by 2 independent cardiologists.

Results: Of the 2,140 consecutive coronary artery disease patients screened, 361 met inclusion criteria, and 174 patients (64.2 ± 7.6 years) completed the study protocol. Of these, 86 had the diagnosis of type 2 diabetes. Among diabetic patients, 62 (72 %) manifested an improvement in ischemic parameters consistent with ischemic preconditioning, whereas among nondiabetic patients, 60 (68 %) manifested ischemic preconditioning ($p = 0.62$). The analysis of patients who demonstrated ischemic preconditioning showed similar improvement in the time to 1.0 mm ST deviation between diabetic and nondiabetic groups (79.4 ± 47.6 vs 65.5 ± 36.4 s, respectively, $p = 0.12$). Regarding rate pressure-product, the improvement was greater in diabetic compared to nondiabetic patients (3011 ± 2430 vs 2081 ± 2139 bpm x mmHg, respectively, $p = 0.01$).

Conclusions: In this study, diabetes mellitus was not associated with impairment in ischemic preconditioning in symptomatic coronary artery disease patients. Furthermore, diabetic patients experienced an improvement in this significant mechanism of myocardial protection.

Keywords: Ischemic preconditioning, Myocardial ischemia, Coronary artery disease, Diabetes mellitus

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BioMed Central

Background

According to the World Health Organization, diabetes mellitus (DM) is one of the most common chronic diseases in the world, affecting 9.3 % of all adults older than 25 years [1]. Moreover, the prevalence of diabetes has been substantially increasing during recent decades [2]. Diabetes poses a high risk for the development of cardiovascular diseases (CVD), because it heralds an accelerated process of atherosclerosis and an increased risk for atherothrombotic complications [3]. CVDs are the leading causes of death in diabetic patients [4] and the substantial rise in diabetes prevalence will ultimately lead to an increase in the burden of diabetes-related CVD in coming decades.

Among the cardiovascular diseases, ischemic heart disease is the most frequent [5, 6] and is associated with the highest morbidity and mortality in patients with type 2 DM.

Despite the advances in the treatment of coronary artery disease (CAD), morbidity and mortality are still high, especially in patients with type 2 DM [7]. Moreover, despite the evolution in the treatment of hyperglycemia, patients with both CAD and diabetes have worse clinical outcomes, irrespective of the treatment applied [8].

Although DM alters the healthy functioning of arteries [9] and blood compounds [10], some clinical studies suggest that myocardial responses to ischemic insults may be deficient in diabetic patients [11, 12], leading to higher myocardial damage and risk of complications. Thus, recent studies [13] have focused on the understanding of such myocardial responses, aiming at discovering the mechanisms of myocardial protection to achieve less aggression and a better prognosis.

A substantial effort has been put forth to investigate any promising cardioprotective strategy to effectively reduce myocardial infarct size. And this matter is of particular importance to diabetic CAD patients [14]. By 1986, in a landmark experimental study, Murry and colleagues demonstrated that a short antecedent period of ischemia could result in a great reduction in myocardial infarct size [15]. For the first time, a method for reducing myocardial cell death other than reperfusion had been discovered. Since its discovery, this phenomenon, termed ischemic preconditioning (IP) has been extensively studied.

Currently, IP is the intrinsic myocardial mechanism that provides the greatest protection regarding the reduction in myocardial ischemic damage. It has demonstrated a 75 % reduction in the infarcted area in animal models that have undergone the preconditioning protocol [15].

IP is assumed as a mechanism of myocardial protection in which brief episodes of sublethal myocardial ischemia followed by reperfusion trigger multiple intracellular pathways that ultimately result in greater myocardial resistance to a subsequent intense ischemic injury. Experimentally, this phenomenon was demonstrated by the reduction in the infarcted area by short episodes of ischemia prior to a pronounced ischemic insult [15].

Although IP was initially thought to result from the opening of collateral vessels and higher coronary flow [16], some interesting studies have shown that the phenomenon occurs irrespective of coronary flow changes [17, 18]. Such studies also demonstrated that IP can be observed by sequential exercise tests (SETs), in which the improvement in ischemic parameters in the second of 2 SETs were confirmed by invasive measurements of myocardial oxygen consumption. Moreover, the clinical observation that patients with chronic ischemic heart disease frequently describe attenuation or even cessation of angina symptoms if they rest and restart the exercise is termed "warm-up" or "walk-through" angina. This phenomenon has been related to IP and documented in studies using SETs [19, 20].

Following the demonstration of IP, the cellular mechanisms underlying the phenomenon began to be investigated. Although some cellular pathways have not been fully elucidated, there is a growing understanding of some phenomena and currently it is assumed that IP may be modulated by external factors like age [21], diseases [22–24], as well as some specific classes of medications [25–27].

Currently, the opening of K-ATP channels plays a fundamental role in the cellular cascades of IP [28]. It is also assumed that medications that bind to this channel may interfere with the IP mechanism. Thus, some classes of medications like the oral hypoglycemic agents may also block such channels in extrapancreatic sites, such as in the heart. It has been shown by some authors that these medications may cause the blockage of myocardial IP [29].

However, despite the fact that some oral hypoglycemic agents may interfere with IP, it is still uncertain whether the intrinsic, complex, intracellular alterations of diabetes itself may affect the cellular mechanisms of this cardioprotective phenomenon. Experimental studies aimed at studying this question have shown conflicting results [30]. Some of these studies have shown that diabetes does not interfere with IP [31, 32], but others have shown negative influences [33, 34]. The great variability in these results is especially due to differences in study protocols, most notably the variability in animal models studied, in the protocols to induce diabetes, the duration of the disease, as well as the variability in myocardial injury protocols. On the other hand, studies in humans are scarce and their results have also been conflicting [12, 13, 35]. Thus, in this study, we aimed at identifying IP in symptomatic multivessel CAD patients and compared the results among patients with and without type 2 DM.

Methods

Study design

This was a prospective study that included patients with symptomatic CAD followed by the Medicine, Angioplasty,

or Surgery Study (MASS) research group. The study design and protocol have been published elsewhere [36]. Briefly, patients were enrolled who had angina symptoms, multivessel CAD, preserved left systolic ventricular function, and a recent, positive ischemic treadmill stress test. All patients gave written informed consent. The diagnosis of type 2 DM was based on American Diabetes Association guidelines diagnostic criteria [37]. Multivessel CAD was confirmed by the finding on cineangiocoronariography of atherosclerotic stenosis of at least 70 % obstruction in 2 or more coronary artery territories. The systolic ventricular function was measured by transthoracic echocardiography and was considered preserved if ejection fraction was ≥ 0.50. Treadmill exercise tests were considered positive for myocardial ischemia if a horizontal or downsloping ST-segment deviation was ≥ 1.0 mm, associated or not with thoracic discomfort. Exclusion criteria were single-vessel CAD, left main CAD, impaired systolic ventricular function (defined as an ejection fraction < 0.50), recent and negative treadmill exercise test, high-risk positive treadmill exercise test, limiting angina symptoms, an acute coronary syndrome in the prior 3 months, electrocardiographic signs that could make difficult the interpretation of ischemic changes (left bundle-branch block, ST-segment deviation), arrhythmias like atrial fibrillation or flutter, severe valvular disease, cardiomyopathies, or patient refusal to participate in this study. The Ethics Committee of the Heart Institute approved this study. All procedures were in accordance with the terms of the Declaration of Helsinki.

Patients
After cardiologic evaluation, all patients were instructed to stop medications with cardiovascular properties 5 days before SETs. Diabetic patients were also instructed to stop antidiabetes medications 5 days before the tests. Only nitrates were allowed until 12 h before the tests. Patients were also recommended to not perform physical activities during the period without the use of medications and were under appropriate nutritional control. A telephone contact was available 24 h a day in case of questions or worsening symptoms. Before treadmill test initiation, a medical team reassessed the symptoms to ascertain clinical stability.

Sequential treadmill exercise tests
All patients underwent 2 SETs, symptom limited, with a 30-min interval between them. The modified Bruce protocol was applied. Tests were conducted during the same period each day, 1 h after lunchtime, on the same treadmill, (MAT 2100 treadmill and a Fukuda Denshi ML8000 Stress Test system (Fukuda Denshi; Bunkyo-ku, Tokyo, Japan).

A 12-lead electrocardiogram, heart rate, and arterial blood pressure were obtained with the patient in the
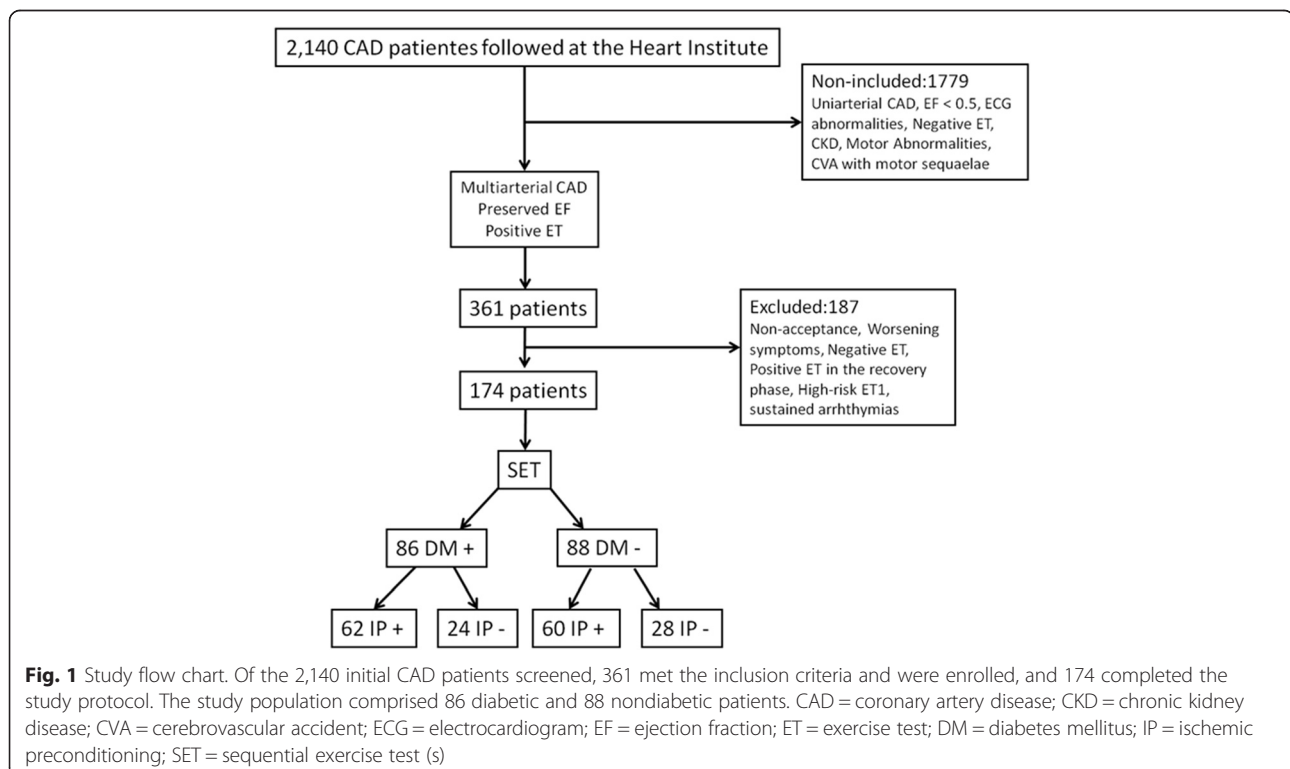

Fig. 1 Study flow chart. Of the 2,140 initial CAD patients screened, 361 met the inclusion criteria and were enrolled, and 174 completed the study protocol. The study population comprised 86 diabetic and 88 nondiabetic patients. CAD = coronary artery disease; CKD = chronic kidney disease; CVA = cerebrovascular accident; ECG = electrocardiogram; EF = ejection fraction; ET = exercise test; DM = diabetes mellitus; IP = ischemic preconditioning; SET = sequential exercise test (s)

Table 1 Main demographic, biochemical, and clinical characteristics of the study population

Total $N = 174$	Diabetics ($n = 86$)	Non-diabetics ($n = 88$)	p Value
Age	64.1 ± 6.8	64.3 ± 8.6	0.84
Male n (%)	73 (84.9)	76 (86.3)	0.83
Hypertension	64 (80)	74 (84.1)	0.54
Smokers	7 (8.8)	7 (8.6)	
Previous smokers	38 (48.1)	33 (40.7)	0.6
Non-smokers	34 (43.0)	41 (50.6)	
Previous AMI	36 (46.1)	17 (22.7)	0.004
CABG	28 (34.6)	23 (26.1)	0.25
PCI	24 (30.4)	28 (32.2)	0.87
EF	0.61 ± 0.06	0.62 ± 0.06	0.2
Tri-vessel disease	38 (52)	43 (51.2)	1.0
Bi-vessel disease	35 (48)	41 (48.8)	1.0
LAD disease	63 (87.5)	74 (86.0)	1.0
Collateral circulation	34 (50.7)	38 (46.9)	0.64
Collateral grade 2/3	30 (88.2)	32 (84.2)	0.74
Weight (kg)	74.5 ± 11.0	73.2 ± 12.7	0.51
Height (m)	1.66 ± 8.1	1.66 ± 9.1	0.75
BMI	26.5 ± 2.8	26.4 ± 3.2	0.54
Fasting glycemia	143.5 ± 47.0	99.0 ± 9.4	0.0001
A1C	7.35 ± 1.61	5.63 ± 0.30	0.0001
BUN	41.9 ± 13.4	40.1 ± 9.4	0.32
Creatinine	1.04 ± 0.23	1.07 ± 0.20	0.37
Total cholesterol	156.5 ± 34.5	168.9 ± 36.3	0.02
LDL cholesterol	90.5 ± 27.4	102.0 ± 32.7	0.01
HDL cholesterol	38.0 ± 9.6	40.3 ± 9.0	0.12
Triglycerides	147.2 ± 88.3	132.6 ± 72.3	0.24

Data are expressed as means ± standard deviation or as absolute and relative risks

AMI stands for acute myocardial infarction, *CABG* coronary artery bypass surgery, *PCI* percutaneous coronary intervention, *EF* ejection fraction, *LAD* left anterior descending, *BMI* body mass index, *A1C* glycosylated hemoglobin, *BUN* blood urea nitrogen, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

standing position at baseline. A 12-lead electrocardiogram was also obtained at each 1.0-min interval during exercise, at peak exercise, each minute up to 5 min during the recovery phase, at the onset of 1.0 mm ST-segment depression, during arrhythmias, and when it was clinically relevant.

A computer-assisted electrocardiogram was monitored during the exercise and recovery phases. The level of the ST-segment deviation was based on visual assessment of the 0.08 s after the J point by 2 independent cardiologists. In case of disagreement, a third cardiologist was consulted, and the matter was resolved by consensus. Only the horizontal or downsloping ST-segment deviations were considered for the time to onset of 1.0-mm ST-segment depression evaluation (T-1.0 mm). Criteria for interrupting the exercise test were ST-segment depression ≥ 3.0 mm, ST segment elevation ≥ 2.0 mm, maximum age-related heart rate, severe arterial hypotension or hypertension, severe chest pain, physical exhaustion, and sustained arrhythmias.

The following parameters were systematically measured: resting heart rate and arterial blood pressure, heart rate and arterial blood pressure at peak exercise, T-1.0 mm in seconds, rate pressure product (RPP) at the onset of T-1.0 mm, and exercise duration in seconds.

The improvement in ischemic parameters (T-1.0 mm and RPP) in the second exercise test compared to the first one indicated the presence of IP. Both tests were blinded for the cardiologist's analysis.

Other parameters recorded during SETs were the total exercise time, the occurrence and density of supraventricular and ventricular arrhythmias, and ST-segment deviation morphology, during exercise and recovery phases. Both arrhythmias and ST-segment morphology were graded according to their density and severity.

According to the levels of fasting glycemia and glycosylated hemoglobin from baseline, patients were separated into quartiles. For fasting glycemia, the quartiles were defined as Quartile 1: < 90 mg/dL, Quartile 2: 90–100 mg/dL, Quartile 3: 101–125 mg/dL, and Quartile 4: ≥ 126 mg/dL. For glycosylated hemoglobin, the quartiles were defined as Quartile 1: < 5.7 %, Quartile 2: 5.7–6.3 %, Quartile 3: 6.4–6.9 %, and Quartile 4: ≥ 7.0 %. The prevalence of patients who expressed IP was compared among the quartiles.
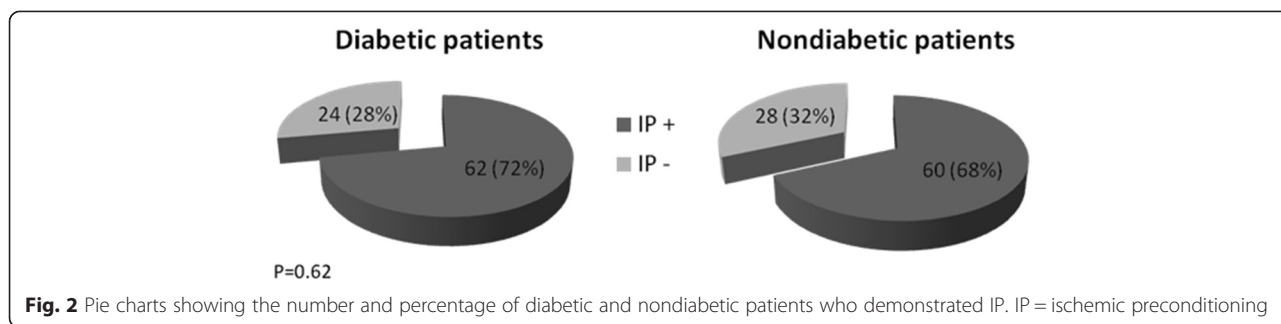
Sample size calculation

The sample size calculation has been previously published elsewhere [36]. Briefly, it was determined by the analysis of studies with similar methodologies that included only diabetic patients [38] and studies that included only nondiabetic patients [21]. By the differences in T-1.0 mm in diabetic and nondiabetic patients, and accounting for an expected loss of 30 % of patients who do not demonstrate the IP phenomenon (estimated

Table 2 Classes of medications used by the two groups of diabetic and nondiabetic patients

	Aspirin / Clopidogrel	Statins	Beta-blockers	ACEI / ARB	Calcium blockers	Diuretics	OAD	Insulins
DM	93.2 %	93.2 %	89.8 %	78.4 %	35.2 %	37.5 %	88.5 %	26.5 %
Non-DM	96.5 %	96.5 %	86.2 %	78.2 %	36.8 %	34.5 %	-	-

DM stands for diabetic patients, *Non-DM* nondiabetic patients, *ACEI* angiotensin converting enzyme inhibitors, *ARB* angiotensin receptor blockers, *OAD* oral antidiabetic drugs

Fig. 2 Pie charts showing the number and percentage of diabetic and nondiabetic patients who demonstrated IP. IP = ischemic preconditioning

based on the experience of our research group), 70 patients with diabetes and 70 without diabetes should be included to test the null hypothesis that the population means were similar (power 0.9 and alpha error 0.05).

Statistical analysis

Continuous variables were compared using an unpaired Student's t test or Mann–Whitney test, when appropriate. The Kolmogorov-Smirnov test was used to assess the distribution of continuous variables. Discrete variables were compared using chi-squared test. Fisher's test was used when appropriate. Data are expressed as mean ± standard deviation or as absolute frequencies and percentages. The software SPSS, version 17.0, was used for all statistical analyses. All tests were 2-sided, and a value of $p < 0.05$ was considered significant.

Results

Of 2,140 patients with stable CAD followed at our institution, 361 met the inclusion criteria. Of those, 187 did not complete the study protocol. The main reasons for non-inclusion and exclusion are shown in Fig. 1. Thus, a total of 174 patients completed the 2 SETs and had IP assessed. The study population comprised 86 patients with DM and 88 without this diagnosis (Fig. 1).

The main demographic, clinical and biochemical characteristics of the 2 groups are presented in Table 1. Despite

the higher prevalence of previous myocardial infarction in the diabetic population and lipid profile (higher LDL-cholesterol levels in nondiabetic patients), both groups had homogeneous characteristics. The duration of diabetes was 11.5 ± 8.8 years (mean ± SD), with a median of 10 years (interquartiles ranges of 6 and 15 years). Table 2 shows the medications used in the two groups of patients.

Demonstration of IP

Among the 86 diabetic patients, 62 (72 %) had an improvement in T-1.0 mm consistent with IP. Among the 88 nondiabetic patients, 60 (68 %) had an ischemic improvement consistent with IP (Fig. 2, $p = 0.62$).

The T-1.0 mm results demonstrated that diabetic patients who demonstrated IP had an improvement in T-1.0 mm between the 2 SETs of 79.4 ± 47.6 s, whereas nondiabetic patients who demonstrated IP had an improvement of 65.5 ± 36.4 s (Table 3, $p = 0.12$).

Regarding RPP, the group of diabetic patients who demonstrated IP had an improvement of $3,011 \pm 2,430$ bpm x mmHg, whereas nondiabetic patients had an improvement of $2,081 \pm 2,139$ bpm x mmHg (Table 4, $p = 0.01$).

Table 5 shows the hemodynamic parameters heart rate and blood pressure at baseline and peak exercise in the 4 groups of patients.

Table 3 T-1.0 mm in exercise test 1 (ET1) and exercise test 2 (ET2) and the difference between the 2 tests (ET2-ET1) in diabetic and nondiabetic patients who demonstrated IP (IP+) or who did not demonstrate IP (IP-)

	ET1	ET2	ET2-ET1	p Value*
DM	269.2 ± 117.8	320.9 ± 132.3	51.7 ± 63.2	0.15
Non-DM	275.6 ± 111.9	314.6 ± 126.2	39.0 ± 52.3	
DM / IP+	274.8 ± 102.8	354.3 ± 115.1	79.4 ± 47.6	0.12
Non-DM / IP+	296.9 ± 107.7	362.4 ± 108.5	65.5 ± 36.4	
DM / IP -	254.7 ± 151.6	234.7 ± 137.0	−20.0 ± 36.4	0.80
Non-DM / IP -	230.1 ± 108.7	212.4 ± 98.2	−17.7 ± 31.9	

Data are expressed as means ± standard deviation. *DM* stands for diabetes mellitus, *IP* + ischemic preconditioning present, *IP* - ischemic preconditioning absent, *ET*, exercise test. * The *p* values are from the comparison of ET2-ET1 among diabetic and nondiabetic patients

Table 4 RPP in exercise test 1 (ET1) and exercise test 2 (ET2) and the difference between the 2 tests (ET2-ET1) in diabetic and nondiabetic patients who demonstrated IP (IP+) or who did not demonstrate IP (IP-)

	ET1	ET2	ET2-ET1	p Value*
DM / IP+	22,841 ± 4,500	25,853 ± 4,742	3,011 ± 2,430	0.01
Non-DM / IP +	23,094 ± 5,312	25,175 ± 5,485	2,081 ± 2,139	
DM / IP -	22,705 ± 4,000	22,124 ± 3,686	−580 ± 2,250	0.43
Non-DM / IP -	23,910 ± 5,380	22,869 ± 5,351	−1,050 ± 2,027	

Data are expressed as means ± standard deviation. *DM* stands for diabetes mellitus, *IP* + ischemic preconditioning present, *IP* - ischemic preconditioning absent, *ET* exercise test. * The *p* values are from the comparison of ET2-ET1 among diabetic and nondiabetic patients

Table 5 Hemodynamic parameters, heart rate and blood pressure in the 4 groups of patients, diabetic and nondiabetic, according to the demonstration of IP

	Variables	ET 1	ET 2	ET2-ET1
DM / IP +	HR (baseline)	79.0 ± 15.1	86.3 ± 17.0	7.3 ± 11.0
	HR (peak)	139.0 ± 15.1	142.1 ± 14.7	3.2 ± 5.6
	BP (baseline)	156.5 ± 18.2	149.7 ± 19.3	−6.8 ± 12.6
	BP (peak)	192.0 ± 25.0	191.8 ± 23.9	−0.16 ± 15.4
DM / IP -	HR (baseline)	79.0 ± 14.7	84.4 ± 14.9	5.4 ± 8.0
	HR (peak)	141.7 ± 14.3	143.0 ± 13.5	1.3 ± 7.4
	BP (baseline)	153.3 ± 22.6	147.1 ± 19.7	−6.2 ± 14.7
	BP (peak)	183.7 ± 23.2	180.6 ± 22.2	−3.12 ± 14.7
Non-DM / IP +	HR (baseline)	78.0 ± 13.1	87.1 ± 13.9	9.1 ± 7.9
	HR (peak)	139.6 ± 15.4	144.3 ± 14.7	4.7 ± 5.7
	BP (baseline)	146.7 ± 20.6	138.7 ± 18.3	−8.0 ± 11.3
	BP (peak)	193.5 ± 23.6	190.2 ± 24.5	−3.3 ± 12.4
Non-DM / IP -	HR (baseline)	82.5 ± 12.9	87.1 ± 12.6	4.6 ± 7.1
	HR (peak)	139.3 ± 12.4	139.6 ± 13.0	0.35 ± 5.2
	BP (baseline)	158.6 ± 26.2	147.9 ± 26.1	−10.7 ± 14.6
	BP (peak)	204.6 ± 25.3	194.8 ± 28.1	−8.9 ± 16.5

Data are expressed as means ± standard deviation. *DM* stands for diabetic patients, *Non-DM* nondiabetic patients, *IP +* ischemic preconditioning present, *IP -* ischemic preconditioning absent, *ET* exercise test

Analyses of secondary ergometric variables

The improvement in the total exercise time comparing the 2 SETs was similar between the groups of diabetic and nondiabetic patients (20 ± 39 s versus 17 ± 36 s, respectively, $p = 0.60$).

The improvement in the frequency and severity of arrhythmias had similar results among diabetic and nondiabetic patients who experienced IP (50 % versus 63 % of patients demonstrated improvement in arrhythmias, respectively; $p = 0.41$).

The improvement in the ST-segment deviation morphology was also similar among diabetic and nondiabetic patients (38.5 % versus 48.3 %, respectively, $p = 0.41$).

Analysis of glycemic variables

When the total group of patients (n = 174) was stratified according to the demonstration of IP, the frequency of diabetic patients as well as the levels of fasting glycemia and glycosylated hemoglobin were similar between the 2 groups, as shown in Table 6.

In addition, when we stratified patients by quartiles of glycemia and glycosylated hemoglobin, there was no statistical difference in terms of IP demonstration among the different quartiles (Fig. 3).

Discussion

Considering that diabetes is an independent risk factor for the occurrence of major cardiovascular events and

Table 6 Frequency of diabetes mellitus, and levels of fasting glycemia and A1c in the study population according to the expression of IP

	IP + (n = 122)	IP - (n = 52)	p Value
DM n (%)	62 (50.8)	24 (46.1)	0.57
Fasting glycemia	121.3 ± 42.6	118.9 ± 34.1	0.72
A1c	6.63 ± 1.6	6.32 ± 1.2	0.25

Data are expressed as means ± standard deviation and as absolute and relative frequencies
DM stands for diabetes mellitus, *A1c* glycosylated hemoglobin, *IP +* ischemic preconditioning present, *IP -* ischemic preconditioning absent
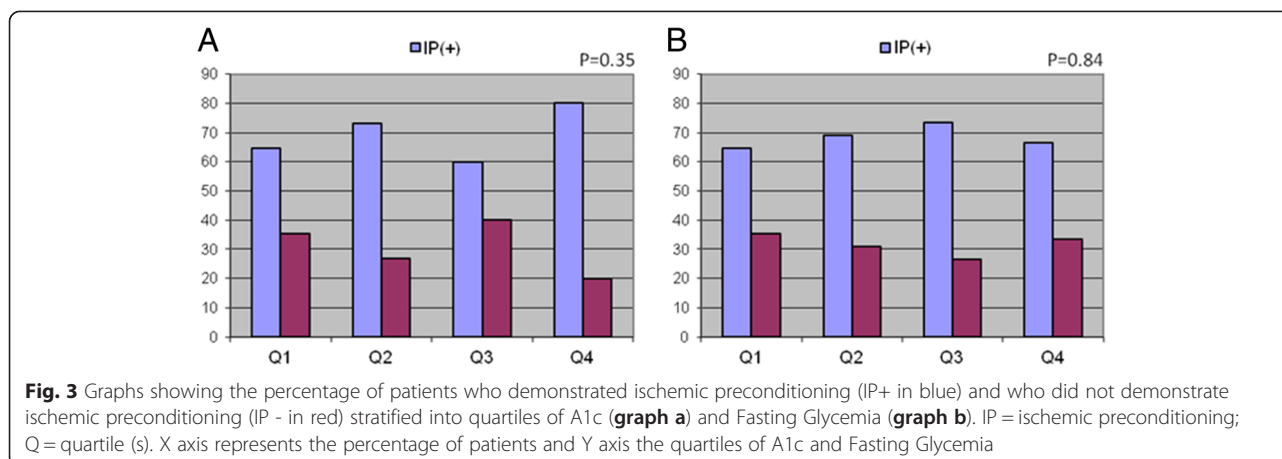
mortality [5, 6], it is reasonable to consider that diabetes might damage the protective mechanism of IP in CAD patients, leading to worse outcomes. However, in this study, the presence of type 2 DM did not have any deleterious effects on the myocardial protective mechanism termed ischemic preconditioning.

In this context, our study showed that patients with type 2 DM demonstrated IP in similar frequency and intensity compared with nondiabetic patients and, interestingly, our data indicated an improvement in ischemic parameters associated with diabetes. The analyses of the data showed a better ischemic response evaluated by RPP in diabetic patients. Thus, this study adds clinical information on some questions that have emerged from contradictory experimental studies.

The improvement in myocardial oxygen consumption, observed by the analysis of RPP, was more pronounced in patients with compared to those without diabetes, indicating better adaptation of the myocardium of diabetic patients after an ischemic insult. Moreover, diabetic patients had an improvement in T-1.0 mm greater than that in nondiabetic patients, although it did not reach statistical significance. Analyzes of the total exercise time also confirmed the main results of the study, as times were similar among patients with and without DM. Similarly, the ST-segment deviation morphology did not differ among diabetic and nondiabetic patients.

Because myocardial ischemia is a frequent cause of arrhythmias during treadmill stress tests, we also assessed the occurrence and complexity of arrhythmias and their improvement during SET. Thus, the improvement in the occurrence of arrhythmias also confirmed the main findings of the study, as the occurrence did not differ among diabetic and nondiabetic patients.

Interestingly, analyzes of the percentage of patients who demonstrated IP among different quartiles of fasting glycemia and glycosylated hemoglobin showed similar rates of IP demonstration. Thus, despite differences in glycemic control, patients demonstrated IP in similar proportions. This analyzes infer that the differences in the intensity of glycemic control in our population did not prevent the demonstration of this cardioprotective phenomenon.

Fig. 3 Graphs showing the percentage of patients who demonstrated ischemic preconditioning (IP+ in blue) and who did not demonstrate ischemic preconditioning (IP - in red) stratified into quartiles of A1c (**graph a**) and Fasting Glycemia (**graph b**). IP = ischemic preconditioning; Q = quartile (s). X axis represents the percentage of patients and Y axis the quartiles of A1c and Fasting Glycemia

However, despite our consistent findings, the information from this study is contrary to Lee's et al. [11] and Ishihara's et al. [12], who have also studied IP in humans.

Lee et al. [11] studied diabetic and nondiabetic CAD patients during percutaneous coronary interventions, and evaluated the action of hypoglycemic agents on IP. They enrolled patients with CAD and formal indication for coronary intervention. Patients underwent consecutive balloon coronary inflations. During the second sequential balloon inflation, patients had less thoracic discomfort, less myocardial lactate production, and lower ST-segment deviation. Moreover, the authors observed that diabetic patients treated with glimepiride had higher lactate production compared to nondiabetic patients treated with glimepiride. Despite this finding, an important limitation of this study is that there was not a direct comparison of diabetic and nondiabetic patients who had no drug interference in IP evaluation.

Ishihara et al. [12] studied patients hospitalized due to an acute myocardial infarction and evaluated the effects of preinfarct angina, on the release of cardiac markers of necrosis, ventricular function, and in-hospital death and compared the results among diabetic and nondiabetic patients. The authors found that in the nondiabetic population, patients with preinfarct angina had a lower release of cardiac markers of necrosis, better recovery of ventricular function, and lower in-hospital mortality, compared to patients with no preinfarct angina. On the other hand, when they analyzed diabetic patients, these variables were not different among patients with and without preinfarct angina. Thus, the authors inferred that diabetes prevented the appearance of IP. Despite their findings, Ishihara's study has important limitations. First, this was a retrospective study, which included a small number of diabetic (n = 121) compared to nondiabetic patients (n = 490). In addition, among the 121 diabetic patients, 53 were taking hypoglycemic medications. Many studies have shown that some of these drugs may

block IP [39], and it has been speculated that this interference with IP mechanisms may partially explain the worse prognosis of diabetic patients hospitalized due to an acute myocardial infarction [40].

On the other hand, other experimental studies that evaluated "in vitro" human myocardial tissue [41, 42] showed results that match those of the present study.

Ghosh et al. [41] studied human atrial appendages from diabetic and nondiabetic patients after a severe ischemic insult. They evaluated the release of cardiac biomarkers of necrosis and the percentage of tissue viability. Among other findings, the authors found a similar intensity in myocardial protection among diabetic and nondiabetic patients.

Additionally, Cleveland et al. [42] evaluated the contractile function of isolated right atrial trabeculae from CAD patients, resected during coronary artery bypass graft surgery. The authors observed the improvement in the contractile strength after an ischemic insult. They showed that the diabetic group that underwent the preconditioning stimuli had similar improvement in contractile function compared to the nondiabetic group.

Moreover, a small study conducted by Bilinska and colleagues [35] with diabetic patients treated with glibenclamide, gliclazide, and diet compared the demonstration of IP in these groups with that in nondiabetic patients. Besides the main findings of the study, which were related to the effects of sulfonylureas in the warm-up phenomenon, they showed that the group of diabetic patients on diet (n = 15) had similar improvement in ischemic parameters compared to nondiabetic patients (n = 17). Of note, this was a secondary result, and the small sample size did not permit to make a definitive conclusion.

Study limitations and strengths

This prospective study on IP in humans had a sample size powered enough to compare IP demonstration in 2

populations with matched clinical characteristics and evaluated the possible interference of diabetes on a myocardial protective mechanism. However, some reflection on the main findings is necessary. In this study, the group of patients with diabetes was under strict control of hyperglycemia, and this is observed by the controlled levels of fasting glucose and glycosylated hemoglobin in the group of diabetic patients. Assuming that hyperglycemia may interfere negatively with IP [43], it is possible that the result of this study would be different in a population under less strict hyperglycemic control.

Clinical practice perspectives

Patients with symptomatic myocardial ischemia evolve with diverse prognosis. In this context, the response of the myocardium to an ischemic insult may probably play an important role in prognosis. One mechanism that may interfere with such a prognosis may be the presence of a protective myocardial phenomenon, termed ischemic preconditioning. Because diabetes is an independent risk factor for the occurrence of major cardiovascular events, it is reasonable that it may compromise this cardioprotective mechanism, leading to worse outcomes. Contrary to the initial expectation, the analysis of our data revealed that diabetic patients showed this protective phenomenon similarly to nondiabetic patients. Moreover, diabetic patients had an improvement in hemodynamic parameters. This study permits to demystify that diabetes interferes with myocardial responses to ischemic insults.

Conclusions

In this study, diabetes mellitus did not substantially affect myocardial IP in symptomatic CAD patients. Furthermore, diabetic patients experienced an improvement in this significant mechanism of myocardial protection.

Abbreviations
CAD: Coronary artery disease; CVD: Cardiovascular disease; DM: Diabetes mellitus; IP: Ischemic preconditioning; MASS: Medicine, Angioplasty, or Surgery Study; RPP: Rate-pressure product; SET: Sequential exercise test; T - 1.0 mm: Time to 1.0 mm ST deviation.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
Each one of the authors have performed the following contributions to this work: P.C.R. researched data and wrote the manuscript. R.M.R. and A.H.U. contributed to exercise treadmill tests and drafting of the manuscript. L.M.A.C., T.L.S., C.L.G. and E.G.L. contributed to exercise treadmill tests and data interpretation. C.A.W.S., P.G., M.T. and M.F.S. contributed to patient selection and reviewed the manuscript. W.H. conceived this study, contributed to discussion, and reviewed the manuscript. J.A.F.R. and R.K.F. reviewed the manuscript. W.H. was the responsible for the final approval of the manuscript submitted. All authors read and approved the final manuscript.

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