Comparative Evaluation of Metformin and Liraglutide Cardioprotective Effect in Rats With Impaired Glucose Tolerance

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Original investigation

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Abstract

Background: Impaired glucose tolerance (IGT) increases cardiovascular risk and can enlarge myocardial infarction (MI) incidence and severity. There is lack of information about cardioprotective potential of glucose-lowering drugs in IGT. We aimed to evaluate the sustainability of myocardium to ischemia-reperfusion injury in diabetic and IGT rats and to study cardioprotective action of metformin and liraglutide.

Methods: Type 2 diabetes mellitus (DM) and IGT were modelled in Wistar rats by high-fat diet and streptozotocin+nicotinamide. 4 weeks after rats were divided into 4 groups: DM (without treatment), IGT (without treatment), IGT+MET (metformin 200 mg/kg per os once daily 8 weeks), IGT+LIRA (liraglutide 0.06 mg/kg s.c. once daily for 8 weeks). Control and high-fat diet groups were made for comparison. After 8 weeks ischemia-reperfusion injury of isolated hearts was performed. Hemodynamic parameters were evaluated and MI size was measured by staining of myocardium slices in triphenyltetrazolium chloride solution. Blood glucose level was measured during the study.

Results: Both IGT and DM led to similar worsening of hemodynamic parameters during ischemia-reperfusion period, in comparison with control group. MI size in IGT (56.76 (51.58; 69.07) %) and DM (57.26 (45.51; 70.08) %) groups was significantly large than in control group (42.98 (33.26; 61.84) %) and did not differ between each other. MI size in high-diet group (56.98 (47.11; 62.83) %) was similarly large as in IGT and DM groups ($p>0.05$). MI size in IGT+MET (42.11 (38.08; 71.96) %) and IGT+LIRA (42.50 (31.37; 60.40) %) was smaller than in both DM and IGT groups ($p<0.05$ for multiple comparison). Myocardium damage size did not differ in IGT+MET and IGT+LIRA groups ($p>0.05$). Only LIRA, but not MET administration to IGT rats led to ischemic contracture reduction. Glycemic control was similar satisfactory in IGT, IGT+MET, IGT+LIRA groups.

Conclusions: IGT and DM have similarly pronounced negative influence on hemodynamics and MI size in transient global rat ischemia ex vivo. Obesity development also has negative impact on the myocardial infarct size. Both MET and LIRA have infarct-limiting effect independent on their influence on glucose level. LIRA, but not MET, diminishes ischemic contracture in IGT rats.

Background

Type 2 diabetes mellitus (DM) is one of the leading problems of modern health care. According to the International Diabetes Federation (IDF) Atlas, there are 463 million people with DM in the world in 2019, and their number is growing steadily [1]. In parallel with the progressive increase in the number of patients with DM, the number of people with prediabetes is rapidly increasing. Thus, in 2019, there are almost 374 million people in the world with an identified impaired glucose tolerance (IGT), which means that every thirteenth adult has this variant of impaired carbohydrate metabolism. IDF predicts that by 2045 the number of such persons will increase by almost one and a half times and will amount to more than 548 million [1].

Prediabetes is a variant of glucose metabolism disorder that develops in individuals with a genetic predisposition when exposed to unfavorable environmental factors such as physical inactivity and pathological food addictions [2]. In the absence of non-drug and drug treatment, prediabetes leads to the development of type 2 DM with a high frequency. Thus, from 5 to 10 % of individuals with prediabetes develop DM every year and up to 70 % develop DM during their life [3, 4].

It is well known that the main damage caused by DM is due to the development of chronic and acute complications that lead to the disability and mortality of these patients [5, 6]. Cardiovascular accidents, including myocardial infarction, have leading position in the structure of type 2 diabetic patients' mortality [1, 7, 8]. At the same time, data on the prevalence and clinical manifestations of cardiovascular diseases, including myocardial infarction (MI), in persons with different forms of prediabetes, such as impaired fasting glycemia (IFG) and IGT, are partly contradictory [9, 10], although the most investigations demonstrate the increased cardiovascular risk in prediabetes.

Thus, a meta-analysis published in 2016 by Yuli Huang et al. that included 53 prospective cohort studies with 1 611 339 individuals revealed that comparing with normoglycemia, both IFG and IGT increase the composite cardio-vascular risk for 1.13 and 1.30 times, respectively. Besides, both these disorders enlarge coronary artery disease and all-cause mortality risk with IGT having more pronounced negative influence [9].

Conversely, another meta-analysis of 20 studies showed that IGT increases the risk of cardiovascular disease by more than 1.5 times, compared with the absence of impaired glucose metabolism, on the other hand IFG does not have such negative impact [11]. Similarly, the Funagata Diabetes Study, a 7-year prospective study conducted in the Japanese population, showed that IGT, but not IFG, is a risk factor for cardiovascular disease [12]. The GAMI (Glucose Tolerance in Patients with acute myocardial infarction) study revealed that IGT is a common variant of glucose metabolism disorders among patients with MI [13] and increases the risk of subsequent cardiovascular events in these patients [14].

The Northern Sweden MONICA study showed that the presence of IGT in women is associated with a significantly higher incidence of painless myocardial ischemia, which is reflected in the detection of electrocardiogram (ECG) changes characterizing the painless MI in the anamnesis [15]. Michal Mazurek et al. investigated the relationship between newly diagnosed disorders of glucose metabolism, such as DM, IGT, and IFG, in patients had just undergone MI, and the frequency of deaths during a prospective follow-up period of an average of 3 years. It was shown that DM and IGT have an equally pronounced negative prognostic effect on the frequency of deaths, at the same time, IFG does not lead to an increase in the mortality of patients, compared with the absence of glucose metabolism disorders [16].

Experimental studies evaluating the effect of IGT on functional and morphological changes in the myocardium are rare. Jia-Liang Liang et al. in 2011 showed that rats with IGT, modeled using the pancreatic toxin streptozotocin, have a decrease in myocardial ejection fraction, as well as an increase in the number of cardiomyocytes prone to apoptosis in the absence of modeling of acute myocardial ischemia [17].

We have not found any experimental work in which the volume of myocardial necrosis was investigated under IGT conditions. At the same time, these are the experimental conditions that make it is possible to create a "pure model" of MI and IGT, which allow to study the direct effect of this glucose metabolism disorder on both hemodynamic parameters and the volume of myocardial necrosis, as well as to study the cardioprotective potential of hypoglycemic drugs.

Methods

The objectives of our study were:

- to compare the severity of myocardial damage caused by transient ischemia ex vivo in animals with IGT, experimental type 2 diabetes and in animals without disorders of glucose metabolism.
- to study of the potential cardioprotective effect of metformin (MET) and a glucagon-like peptide-1 (GLP-1) receptor agonist liraglutide (LIRA) in animals with IGT and to compare the prominence of this effect in transient myocardial ischemia ex vivo.

We chose MET as it is the first-line drug for DM therapy [18], which is often used for IGT and has shown its effectiveness in patients with IGT in terms of preventing the development of DM. For GLP-1 receptor agonists, numerous pleotropic effects have been described in recent years, among which the cardioprotective effect occupies one of the leading positions. Thus, LIRA therapy, according to the LEADER study, demonstrated the ability to reduce overall mortality, mainly by reducing cardiovascular mortality [19]. Currently, GLP-1 receptor agonists are recommended for both primary and secondary prevention of cardio-vascular diseases.

Animals

The study was carried out in male Wistar rats weighing 150-250 g (n = 32).

The animals were maintained in fixed light mode, 12.00: 12.00 h (light: dark), no more than 5 animals per cage with free access to food and water. The temperature was maintained within the range of 22-25 ° C, the relative humidity - 50-70%.

The duration of quarantine (acclimatization period) for all animals was 14 days. During the quarantine, every animal was examined daily. The color of the skin and visible mucous membranes, behavior, the motor activity, the presence of seizures, changes in the respiratory movements, and tail position were assessed. Weighing was carried out upon arrival of the animals and during the quarantine period - at least 1 time per week. Animals with deviations in weight, general condition or behavior were not included in the experiment.

Study groups

After the acclimatization period, the following experimental groups were formed:

1. «CRL» (n=6) - control group – rats were fed with standard chow for 16 weeks
2. «HFD» (n=8) - high-fat diet group – rats were fed with high-fat diet for 16 weeks
3. «DM» (n=4) - type 2 diabetes mellitus group – rats with experimental type 2 diabetes mellitus were kept without treatment for 12 weeks
4. «IGT» (n=4) - impaired glucose tolerance group - rats with impaired glucose tolerance were kept without treatment for 12 weeks
5. «IGT+MET» (n=4) - impaired glucose tolerance + metformin group – in 4 weeks after the induction of impaired glucose tolerance started 8-weeks metformin therapy
6. «IGT+LIRA» (n=4) - impaired glucose tolerance + liraglutide group – in 4 weeks after the induction of impaired glucose tolerance started 8-weeks liraglutide therapy

The number of animals in these groups was determined during the experiment, depending on which variant of the carbohydrate metabolism disorder developed after streptozotocin and nicotinamide injection.

The study design is shown in Figure 1.

Induction of type 2 diabetes mellitus and impaired glucose tolerance

Animals were kept on the diet with increased amount of saturated fat (25%) (further: high-fat diet, HFD) during all the experiment.

After first 4 weeks of high-fat diet a solution of nicotinamide (Nicotimamide, Sigma-Aldrich, St. Louis, MO, USA) 230 mg/kg was injected intraperitoneally as a pancreatic protector, after 15 minutes - a solution of streptozotocin (Streptozocin, Sigma-Aldrich, St. Louis, MO, USA) 60 mg/kg intraperitoneally as a pancreatic toxin [20].

On the second and third days after the administration of nicotinamide and streptozotocin, glycemia was determined. For this, a tail vein puncture was performed, after which the glucose content in the obtained venous blood drop was determined using an Accu-Chek Performa glucometer (Roshe, Germany). Glycemic values of 3.3 to 7.8 mmol/L were considered normal, since the measurement was made during the day (not fasting). DM was diagnosed when two measurements performed in different days showed glycemia elevation more than 11.1 mmol/L [21, 22]. If lower glycemic values were found in at least one of the measurements, an oral glucose tolerance test (OGTT) was performed. Glycemia was measured initially (fasting), as well as 15, 30, and 60 minutes after the gastric administration of a 40% glucose solution 3 g/kg of animal body weight. If we detected glycemia more than 11.1 mmol/L at any of the measurement points during OGTT we diagnosed the presence of DM. If glycemia was in the interval of 7.8-11.0 mmol/L we diagnosed IGT [23]. Animals in which the glycemic parameters at all measurement points did not exceed 7.8 mmol/L were regarded as having no disturbances in glucose metabolism and were excluded from further experiment.

Glycemic measurements for making the diagnosis of DM and IGT are shown in Table 1.

Table 1. Blood glucose measurement for DM and IGT diagnostics.

Group	animal №	Blood glucose level, mmol/L						
		1st measurement	2nd measurement	ORAL GLUCOSE TOLERANCE TEST				
				baseline	15 min	30 min	60 min	90 min
DM	1	16.0	12.1					
	2	20.5	21.6					
	3	19.2	16.0					
	4	9.7	8.6	3.8	10.5	17.6	18.0	15.3
IGT	5	9.9	7.7	4.2	7.0	9.3	10.2	10.9
	6	6.5	6.4	4.6	8.9	8.7	10.2	9.2
	7	6.1	6.3	3.8	5.7	7.1	9.5	8.0
	8	7.7	7.0	5.3	5.9	7.9	8.8	9.6
IGT+MET	9	6.8	5.7	4.2	10.1	10.4	9.3	9.9
	10	6.7	6.9	4.5	9.6	10.4	10.6	9.9
	11	5.6	5.8	5.4	7.7	9.2	9.7	9.2
	12	6.7	5.3	5.3	6.6	9.8	10.7	10.8
IGT+LIRA	13	8.6	8.5	4.6	6.9	8.1	7.3	9.0
	14	5.7	6.3	4.7	8.2	8.6	9.5	9.0
	15	5.4	8.0	4.5	7.4	7.7	9.8	8.5
	16	5.3	7.7	4.8	7.3	8.8	10.5	9.6

Study drugs

Metformin powder (Metformin hydrochloride Sigma-Aldrich, St. Louis, MO, USA) was dissolved in distilled water and given per os by gastric tube 200 mg/kg of body weight once daily for 8 weeks (56 days).

Liraglutide (Victoza, NovoNordisk, Denmark) was administered subcutaneously 0.06 mg/kg of body weight once daily for 8 weeks (56 days).

Body weight and food consumption measurement

Once every two days during the entire experiment, the animals were weighed, and the weight of the chow consumed in 2 days was determined.

Glucose measurement

Glucose measurement was performed with the help of Accu-Chek Performa glucometer (Roshe, Germany).

In CRL and HFD groups glucose assessment was performed at the end of the 4^{th} week (28^{th} day), 8^{th} week (56^{th} day), 10^{th} week (70^{th} day), 12^{th} week (84^{th} day), 14^{th} week (98^{th} day) and 16^{th} week (112^{th} day) of experiment at the same daytime (not fasting measurement).

In DM and IGT groups glucose assessment was performed after 4 weeks of experiment on the 2^{nd} and 3^{rd} day after streptozotocin injection, at the end of the 8^{th} week (56^{th} day), 10^{th} week (70^{th} day), 12^{th} week (84^{th} day), 14^{th} week (98^{th} day) and 16^{th} week (112^{th} day) of experiment at the same daytime (not fasting measurement).

In groups IGT+MET and IGT+LIRA groups glucose assessment was performed after 4 weeks of experiment on the 2^{nd} and 3^{rd} day after streptozotocin injection, at the end of the 8^{th} week (56^{th} day), then every third day during the 8 weeks of treatment at the same daytime (not fasting measurement), 5 hours after the certain treatment.

Isolated heart perfusion according to Langendorff

Anesthesia was performed by Zoletil (tiletamine hydrochloride 30 mg/kg and zolazepam hydrochloride 30 mg/kg) intramuscularly and xylazine hydrochloride 6 mg/kg intramuscularly. After reaching the surgical stage of anesthesia (Zoletil + Xylazine IM), a wide thoracolaparotomy was performed, the organs of the chest cavity were exposed and the heart was removed, after which it was connected to the modified Langendorff apparatus. Perfusion was performed retrogradely through the ascending aorta, while venous outflow of perfusate occurred from the right chambers of the heart. A polyethylene balloon was inserted into the left ventricular cavity, connected to a pressure transducer to register intra-left ventricular pressure and create an adequate preload. Coronary perfusion volumetric rate was also recorded by measuring venous outflow. Perfusion was performed with modified Krebs-Henseleit buffer solution (consisting of the following [in mmol/L]: NaCl, 118.5; KCl, 4.7; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11; and $CaCl_2$, 1.5) at a constant pressure of 80 mm Hg and temperature +37 C [24]. In this case, the left ventricle contracted in isovolumetric mode due to the fact that the volume of the balloon introduced into its cavity was constant and provided preload at the physiological level (no more than 12

mm Hg). Intra-ventricular pressure was recorded using PhysExp software (Cardioprotect Ltd., Saint Petersburg, Russian Federation).

After the end of the stabilization period lasting 5 minutes, the functional parameters of the heart were recorded. Left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) were measured isovolumetrically using a nonelastic polyethylene balloon introduced into the left ventricle via the left atrium. Left ventricular developed pressure (LVDP) was calculated as the difference between LVSP and LVEDP. Intensity of coronary perfusion (coronary flow rate (CFR) was determined by measuring the time for the collection of perfusate outflow.

Total 30-minute normothermic myocardial ischemia and subsequent 90-minute reperfusion were induced by reversible shutdown of perfusion. During the period of ischemia, the value of intra-left ventricular pressure was recorded every 5 minutes in order to assess the severity of ischemic contracture. During the reperfusion period, functional parameters (coronary blood flow and intra-ventricular pressure) were recorded every 15 minutes.

Infarction size measurement

At the end of reperfusion, the volume of the irreversibly damaged myocardium was measured by the method of histochemical staining of transverse sections of the heart with 1% triphenyltetrazolium chloride solution. The sections were incubated in the indicated solution for 15 minutes and the viable myocardium was stained bright red. Areas of irreversibly damaged myocardium remained unstained. Then the photographs of the sections were made with a stereomicroscope (SMZ18; Nikon, Tokyo, Japan) coupled to a digital camera (DS-Fi2, Nikon, Tokyo, Japan) and computer processing of the images was performed. Infarction size was expressed as a percentage of total ventricular area minus the cavities.

Statistical analysis

Statistical data processing was performed using the software package IBM SPSS Statistics-22 (IBM, USA) and Statistica-10 (Statsoft, USA).

The significance of differences between groups was assessed using the nonparametric Kruskal-Wallace and Mann-Whitney test for independent samples, using a nonparametric analysis of variance (a posteriori pairwise comparison of groups using the Dunn test). The significance of differences within one group was assessed using the nonparametric Friedman and Wilcoxon tests for dependent variables with the introduction of the Bonferroni correction with false discovery rate. All indicators are presented as "median (25%; 75%)". P values less than 0.05 were considered significant.

Results

Streptozotocin+nicotinamide administration failed to induce any impairment of glucose metsabolism in 2 rats, that were therefore excluded of the further experiment. In 4 rats type 2 DM was observed according

to the above mentioned criteria, 12 rats developed IGT, therefore the latter were divided into 3 groups 4 animals each (“IGT”, “IGT+MET”, “IGT+LIRA”).

Body weight and food consumption

During the first 4 weeks of experiment rats receiving high-fat diet has significantly more intensive weight gain that those fed with standard chow ($p<0.05$) (Fig. 2A, Table S1). After dividing into the study groups, it was primarily observed that weight gain in DM, IGT and HFD groups did not differ significantly among each other ($p>0.05$) and was less prominent than in CRL group ($p<0.05$). But after 8 weeks of experiment rats of IGT group demonstrated more intensive weight gain than those with DM or HFD groups ($p<0.05$ for each). Interestingly, animals with IGT treated with MET had similar weight gain as those with IGT without treatment ($p>0.05$). On the other hand, treatment of IGT rats with LIRA slowed physiological body weight increase (Fig. 2A, Table S1), which reflects the known anorexigenic effect of LIRA [18].

Food consumption in IGT group was similar to that in DM group and significantly exceeded food consumption in both CRL and HFD groups. HFD animals had slightly larger food consumption than CRL ones. MET administration to IGT animals in general did not decrease food intake comparing with IGT without therapy. On the other hand, treatment of IGT rats with LIRA led to significant decrease of chow consumption which was most prominent by the 16 week of experiment (Fig. 2B, Table S2).

Blood glucose level

Animals in DM had higher blood glucose level than animals in CRL, HFD, IGT, IGT+MET and IGT+LIRA groups. Of note, glycemic profile in IGT rats without treatment did not significantly differ from that in the rats with IGT treated with MET or LIRA. Similarly, MET and LIRA administration to IGT rats had comparable glucose-lowering effect, which, in our opinion, might be due to the fact that pre-treatment glucose level almost did not exceed normal range. Glycemia dynamics is shown in Fig. 2C and in Table S3. No hypoglycemic episodes were observed an any of the study groups.

Isolated heart function and myocardial infarct size

Changes in LVP that occurred during the global ischemia are shown in Fig 3A and Table S4. Ischemic contracture was determined as follows: at least a threefold increase in LVP at any time during the ischemic period in comparison to the LVP after five minutes of ischemia. We observed the most prominent increase in left ventricle (LV) pressure in DM and IGT groups after 10 minutes of ischemia, no differences were found between these groups. LV pressure in IGT+MET group was similar to that in CRL and HFD groups and lower than in DM and IGT groups in 10 minutes, but from the 15 minute up to the end of ischemic period LV pressure in IGT+MET group caught up with that in IGT and DM groups. Of note, LV pressure in IGT+LIRA group was significantly lower than in IGT, DM groups and in IGT+MET group during all the ischemic period and did not differ from that in both CRL and HFD groups from 15 to 30 minutes, being even lower at the 10 minute (Fig. 3A, Table S4). Thus, we can conclude that LIRA administration in IGT effectively diminishes ischemic contracture.

The baseline LVDP values were similar in all the groups. LVDP in IGT group tended to be higher than in DM group for 30-60 minutes of reperfusion period, although the differences were not significant ($p>0.05$). Interestingly, LVDP in IGT group was higher than in both CRL and HFD groups. Moreover, LVDP was lower in both IGT+MET and IGT+LIRA groups comparing with IGT and DM groups. No significant differences were observed between IGT+MET and IGT+LIRA groups, although LVDP tended to be lower in IGT+LIRA group, most probably due to lower LVSP (Fig. 3B, Table S5).

There were no significant differences in LVEDP (Fig. 3C, Table S6). Similarly, CFR did not differ in all the groups during reperfusion period, except HFD group, where CFR tended to be higher in 30-45 minutes of reperfusion ($p>0.05$), comparing with all the other groups (Fig. 3D, Table S7).

Myocardium infarct size in DM group was larger than in CRL group (57.26 (45.51; 70.08) % and 42.98 (33.26; 61.84) %, respectively, $p<0.05$). Of note, myocardium damage size was similarly large in both IGT (56.76 (51.58; 69.07) %) and HFD (56.98 (47.11; 62.83) %)) groups as in DM group (57.26 (45.51; 70.08) %), $p>0.05$ for multiple comparison. On the other hand, both in IGT+MET (42.11 (38.08; 71.96) %) and IGT+LIRA (42.50 (31.37; 60.40) %) groups myocardial infarction size was significantly smaller than in IGT or DM group ($p<0.05$). No difference was found between IGT+MET and IGT+LIRA groups ($p>0.05$). Importantly, myocardial damage size in IGT+MET and in IGT+LIRA groups was similar to that in CRL group of animals without any glucose metabolism impairments ($p>0.05$) (Fig. 3E, Table S8).

Discussion

We have demonstrated that both type 2 DM and IGT have a negative effect on hemodynamic parameters and lead to the formation of pronounced structural changes in the myocardium in conditions of transient ischemia ex vivo. It is noteworthy that all hemodynamic parameters, during both ischemia and reperfusion period, coronary blood flow, as well as myocardial damage volume did not differ in animals with DM and IGT, although glycemia parameters in diabetic animals were significantly higher. This allows us to suggest that impairment of glucose metabolism, regardless of the severity of hyperglycemia, has a negative effect on the resistance of the myocardium to ischemic-reperfusion injury. Moreover, feeding with high-fat diet, resulting in obesity development, without glucose metabolism impairment, has similar negative impact on structural changes of the myocardium, as myocardial infarction volume in HFD animals did not differ from that in IGT and DM rats.

Both MET and LIRA have infarct-limiting properties in transient myocardial ischemia in rats with IGT. We did not observe any significant difference in the prominence of infarct-limiting action of these two drugs. The myocardial infarction volume in IGT animals treated with both MET and LIRA was significantly smaller than that in type 2 diabetic animals and in animals with IGT without treatment.

At the same time, only LIRA, but not MET, has a positive influence on hemodynamic parameters, predominantly decreasing ischemic contracture, which might comprise one of mechanisms of LIRA cardioprotective action.

As to our knowledge, our study was the first that evaluated and compared cardioprotective properties of MET and LIRA in rats with IGT. There are a few data elucidating cardioprotective potential of MET in the clinical conditions, in patients with prediabetes and even in individuals without impairment of glucose metabolism [25, 26, 29, 30]. Some of these trials predominantly focus on the evaluation of surrogate markers potentially characterizing cardiovascular risk. Thus, Chris P.H. Lexis et al. investigated the effects of 4-months MET therapy in patients without glucose metabolism abnormalities having undergone MI on cardiovascular risk factors and revealed that MET causes improvement of glycated hemoglobin, total cholesterol, low-density cholesterol, body mass index, comparing with placebo [25].

It has also been demonstrated that patients with stable angina and IGT receiving MET therapy have better prognosis than individuals with stable angina and IGT without treatment which is characterized by lower incidence of major adverse cardiac events in the follow-up observation period [26].

There are some data about cardioprotective properties of MET in animals without glucose metabolism impairments. For example, Hamid Soraya et al. showed that administration of MET for the short period of time while modelling isoproterenol-induced MI in healthy rats causes significant infarct-limiting effect [27]. Nevertheless, we have not found experimental works evaluating infarct-limiting effect of MET in prediabetic conditions. Therefor we may assume that the present study is the first elucidating its cardioprotective potential in transient global myocardial ischemia. On the other hand,

Of note, MET influence on hemodynamic parameters, both in clinical and experimental trails, are contradictory. Thus, diastolic function measured by tissue Doppler imaging in patients with DM was shown to be improved by MET treatment after coronary angiography [28]. Left ventricular end-diastolic pressure was lowered in a non-diabetic rat model of post-MI heart failure [29]. However, in conditions of ST-elevation MI in patients without impairment of glucose metabolism, MET failed to preserve left ventricular ejection fraction at 4 months of treatment [25]. In the meta-analysis including 4 randomized clinical trials involving 1366 non-diabetic patients MET was found to significantly reduce left ventricular ejection fraction and increase both left ventricular end-diastolic and left-systolic volume [30]. These data correlate with the results of our study, where we observed significant infarct-limiting effect of MET although no positive influence on hemodynamic parameters was discovered, including no influence on ischemic contracture and no certain improvement of reperfusion hemodynamics.

Interestingly, it is thought that potential cardioprotective effect of MET might be due to endothelial dysfunction diminishing. Thus, it has been shown that patients with IGT receiving MET therapy have less prominent endothelial dysfunction of left anterior descending coronary artery evaluated by acetylcholine probe during coronary angiography than those with IGT without MET treatment [26]. Earlier we have also investigated endothelial protective effect of MET in comparison with LIRA in patients with type 2 DM [31]. We demonstrated that 9-months treatment with MET improves vasomotor endothelial characterized by decrease of circulating endothelin-1 level, although this action was strongly connected with glycemic profile normalization and was diminished in patients with glycated hemoglobin increase. Moreover, we did not observe any positive effect of MET on endothelial-dependent vasodilation studied by

acetylcholine electrophoresis. Taken together, these data imply that MET does not have its own endothelial-protective property, without connection with its influence on glucose metabolism, in diabetic conditions, although it might have an endothelial protective potential in prediabetes. On the other hand, even in prediabetic conditions we cannot completely exclude the potential connection of MET endothelial protective effect with better glucose profile in use of this drug.

According to the literature data, cardioprotective effect of MET might be connected with adenosine monophosphate activated protein kinase (AMPK) activation. AMPK activates the energy conserving metabolic processes which leads to increased tolerance to any hypoxia, including that taking place during ischemia-reperfusion injury [32]. Regarding myocardium, the AMPK system is prominently activated by hypoxia only under pathological ischemic circumstances, whereas AMPK-activating drugs like MET mimic these effects of hypoxia. The AMPK activation by means of MET causes glucose uptake increase and stimulates glycolysis in the cardiomyocytes and thereby makes the heart more resistant in the future ischemic conditions, serving the aims of pharmacological preconditioning [33]. AMPK activation also suppresses cell growth and proliferation and thereby prevents myocardial remodeling after MI [27], which might be important in the conditions of chronic experiment and could not be realized in our study.

Notably, no clinical trails have been found evaluating the opportunity of LIRA do decrease the incidence and reduce severity of MI or to improve the stable angina development in IGT conditions, though its cardioprotective effect in type 2 DM is well known [19, 34, 35]. It has only been demonstrated that liraglutide 3.0 mg, approved for the treatment of obese patients without DM, reduces incidence of DM development in obese patients with prediabetes. In the SCALE Obesity and Prediabetes study, in IGT patients LIRA 3.0 mg therapy showed high effectiveness in body mass index reduction which might be interpreted as decrease of cardiovascular risk factor [36]. Most probable the reason is that this drug has not been approved for the use in IGT which limits its practical use in this pathology.

Similarly, to the situation observed for MET, no experimental studies elucidating cardioprotective effect of LIRA in myocardial ischemic-reperfusion injury in animals with prediabetes have been found. Therefore, our study seems to by the first one describing the influence of LIRA on hemodynamic parameters and myocardium damage volume in experimental conditions of IGT.

On the other hand, there is a rather big amount of investigations aimed to determine the cardioprotective effect of LIRA in both animals without glucose metabolism abnormalities and with DM.

Thus, the use of LIRA in animals without DM increased the survival rate after MI, decreased the necrosis volume and the severity of cardiac hypertrophy manifestations. Similarly, treatment with LIRA in mice with experimental type 2 DM increased survival compared with placebo, which was not observed with MET, despite similarly satisfactory glycemic control [37].

D.-D. Huang et al. demonstrated that the administration of LIRA to rats without DM after experimental myocardial infarction improves cardiac function and slows down the fibrosis development [38]. The

potential mechanism of LIRA cardioprotective action both in normal glycemic status and in DM is supposed to be connected with endothelial dysfunction diminishing. Thus, it has been shown that LIRA increases NO production, decreases edothelin-1 level, normalizing vasomotor endothelial function, and increases microvasculature blood flow in obese rats [39]. Similarly, the use of LIRA increases nitric oxide level in diabetic rats [40].

Also, it has been described that in DM LIRA protective mechanisms might be due to positive influence on the manifestations of insulin resistance and adiposopathy which is characterized by adiponectin increase. Similar mechanisms might also have an impact it IGT [40].

Interestingly, some of the cardioprotective effects of GLP-1 receptor agonists administration during ischemia/reperfusion injury are preserved in isolated hearts of GLP-1 receptor knock-out mice, suggesting the existence of a GLP-1 receptor independent pathway of cardioprotection. At the same time, administration of GLP-1(9–36), product of GLP-1 degradation by dipeptidylpeptidase-4, to GLP-1 receptor knock-out animals results in ischemic damage reduction, suggesting that GLP-1(9–36) might be an activator of the GLP-1 receptor independent pathway associated with cardioprotection [41].

Conclusions

Thus, we can conclude that IGT and DM have similarly pronounced negative influence on the resistance of myocardium to ischemic-reperfusion injury, regardless the hyperglycemia severity. Moreover, obesity development also has negative impact on the myocardial structural damage in ischemic-reperfusion conditions. Both MET and LIRA have certain cardioprotective effect that is independent on their positive influence on glucose metabolism, for no differences were observed in glycemic control during the use of these two drugs. Cardioprotective property of LIRA includes both improvement of hemodynamic parameters and infarct-limiting action, whereas use of MET only decreases myocardial damage volume. Therefore, we can suppose that cardioprotective action of LIRA is more prominent in transient myocardial ischemia in IGT rats. The received data might open the perspectives for clinical investigation of potential cardioprotective effects of both MET and LIRA in patients with IGT.

List Of Abbreviations

IGT: impaired glucose tolerance

MI: myocardial infarction

DM: diabetes mellitus

MET: metformin

LIRA: liraglutide

IDF: International Diabetes Federation

IFG: impaired fasting glucose

GAMI: “Glucose Tolerance in Patients with acute myocardial infarction” [study]

ECG: electrocardiogram

GLP-1: glucagon-like peptide-1

OGTT: oral glucose tolerance test

HFD: high-fat diet

N/A: nicotinamide

STR: streptozotocin

CRL: control [group]

BL: baseline

LVSP: left ventricular systolic pressure

LVEDP: left ventricular end-diastolic pressure

LVDP: left ventricular developed pressure

CFR: coronary flow rate

LV: left ventricle

AMPK: adenosine monophosphate activated protein kinase

Declarations

Ethics approval

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85–23, revised 1996) and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. The study protocol was approved by Institutional Animal Care and Use Committee of Almazov National Medical Research Centre (Protocol Number 19-1П3#V1, Jan 14 2019). All efforts were performed to protect the laboratory animals and minimize their suffering throughout the study. The experiments complied with the ARRIVE guidelines (http://www.nc3rs.org/ARRIVE).

Consent for publication

Not applicable

Availability of data and materials

The data generated and analyzed during this study are included in this published article and its supplementary information files. Additional information is available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AS made the primary collection and preparation of data, analysed the date, performed statistical analysis and was a major contributor in writing the manuscript.

SM performed all the manipulations with isolated heart and collected primary data concerning ischemia-reperfusion injury and myocardial infarction.

TK developed the study design, coordinated all the steps of this work, reviewed the manuscript.

TV developed the study design, including choice of study drugs doses, duration of treatment, etc., reviewed the manuscript.

NT, OSh, AKh, ASh, VT and DS performed all manipulations in animals, except ischemia-reperfusion modelling. These manipulations included measurement of body weight and food consumption, DM modelling, glucose measurement, OGTT performance, study drugs administration. NT also took part in statistical analysis and preparation of figures for the manuscript.

YuB performed all the investigations, including body weight and food consumption measurements, glucose level assessment, in CRL and HFD groups, and analyzed received data in these groups including hemodynamic parameters and infarction measurement.

MG developed the study design, calculated the MI size, reviewed the manuscript.

All authors read and approved the final manuscript.

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Figures

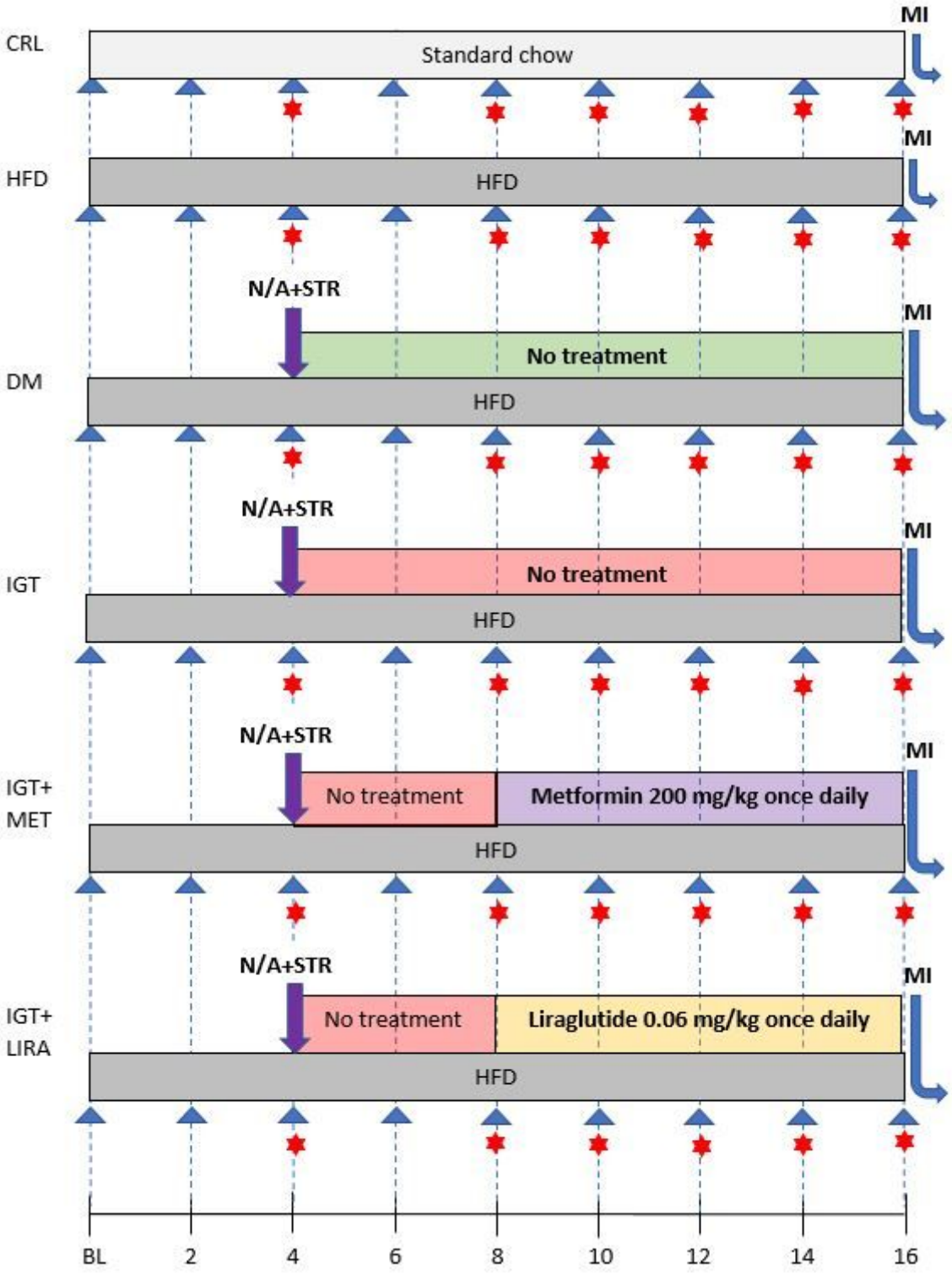
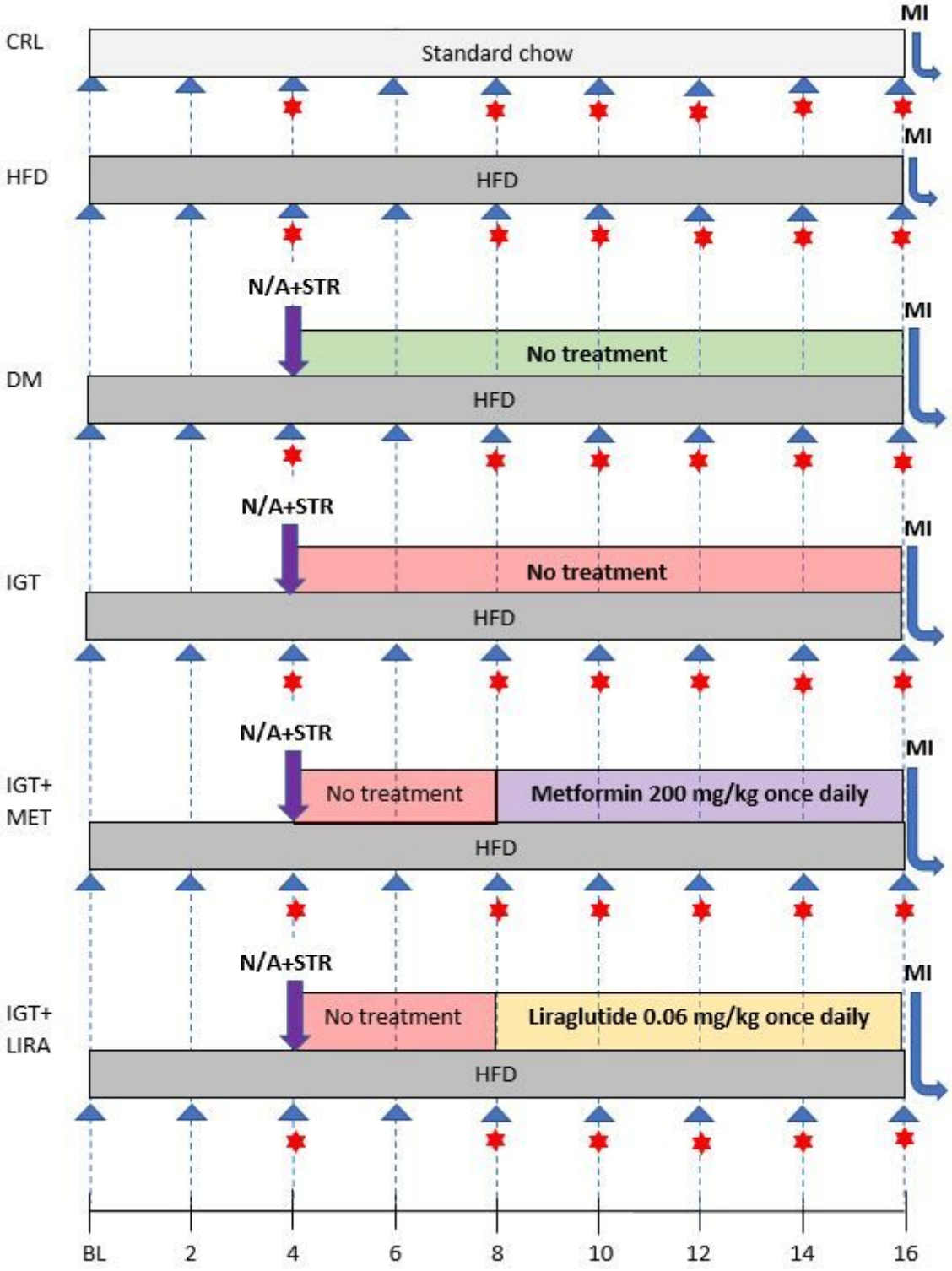


Figure 1

Study design. N/A – nicotinamide, STR – streptozotocin, MI – myocardial infarction modelling, BL - baseline. - body weight and food consumption measurement. – blood glucose measurement



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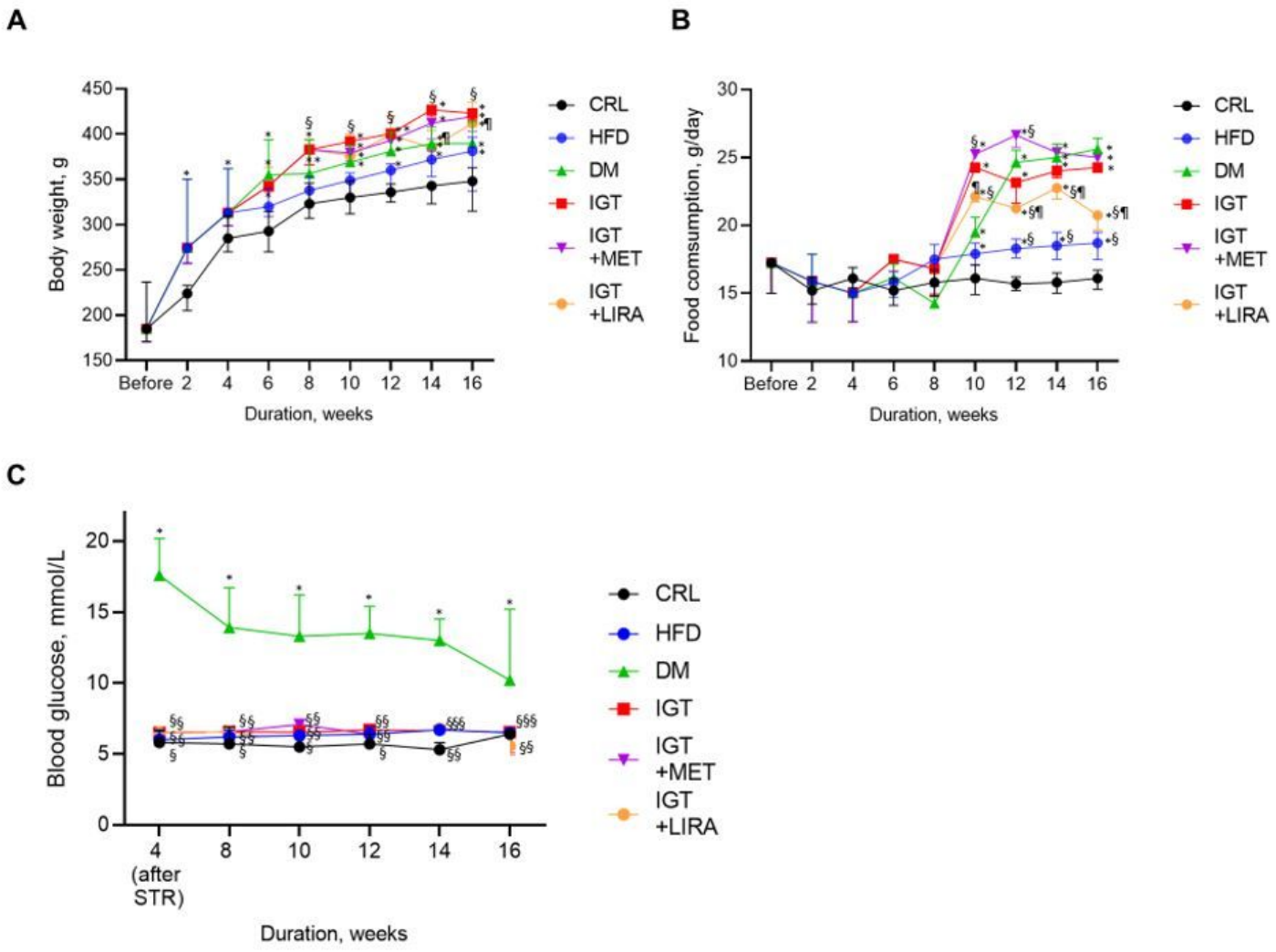
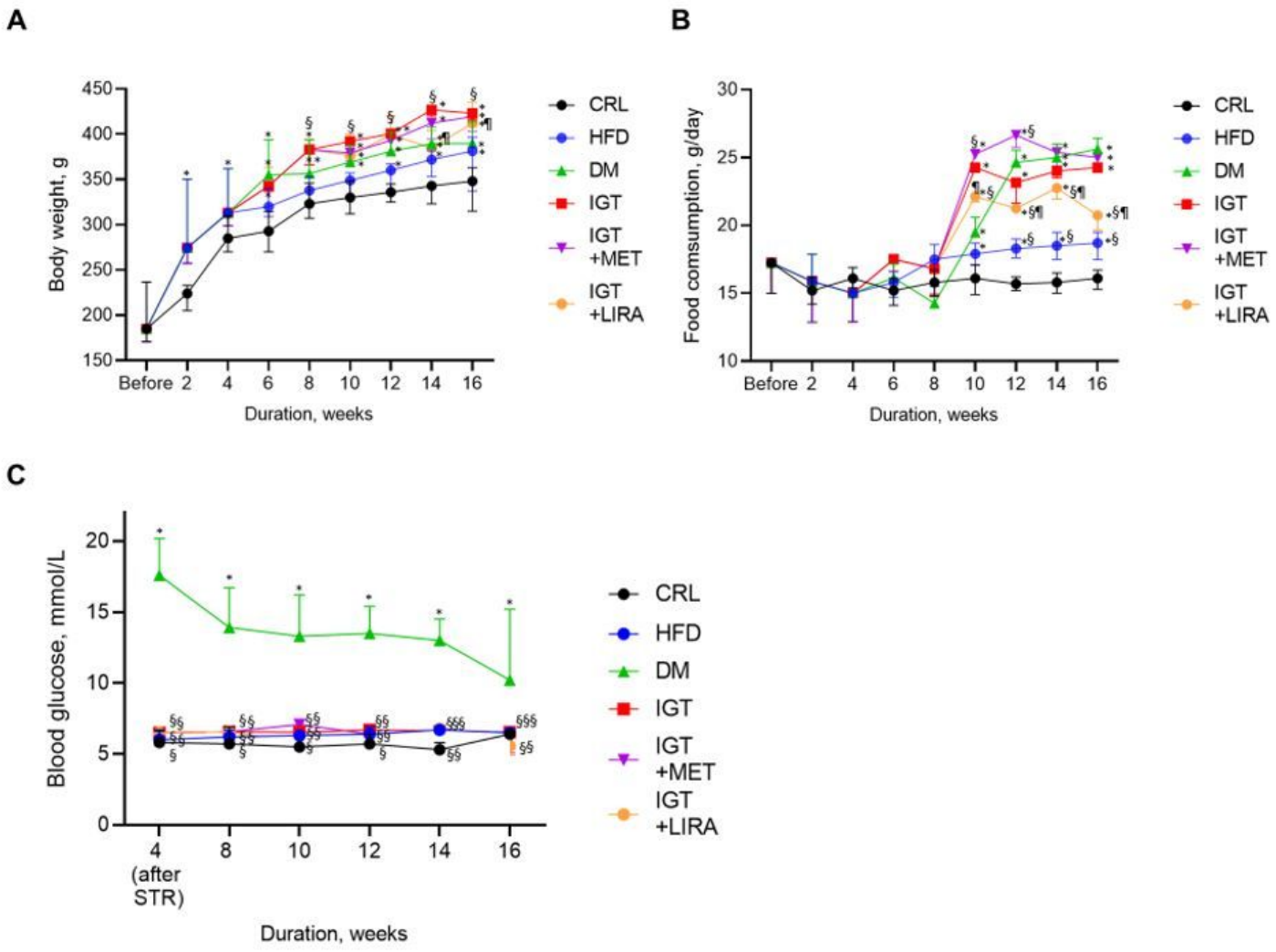


Figure 2

Dynamics in body weight, food consumption and glycemia during the experiment. (A) body weight, (B) food consumption, (C) blood glucose dynamics. Results are presented as median (25; 75) %. *- p<0.05, comparing with CRL group. § - p<0.05, comparing with DM group. ¶ - <0.05, while comparing between groups IGT+MET and IGT+LIRA. STR – streptozotocin



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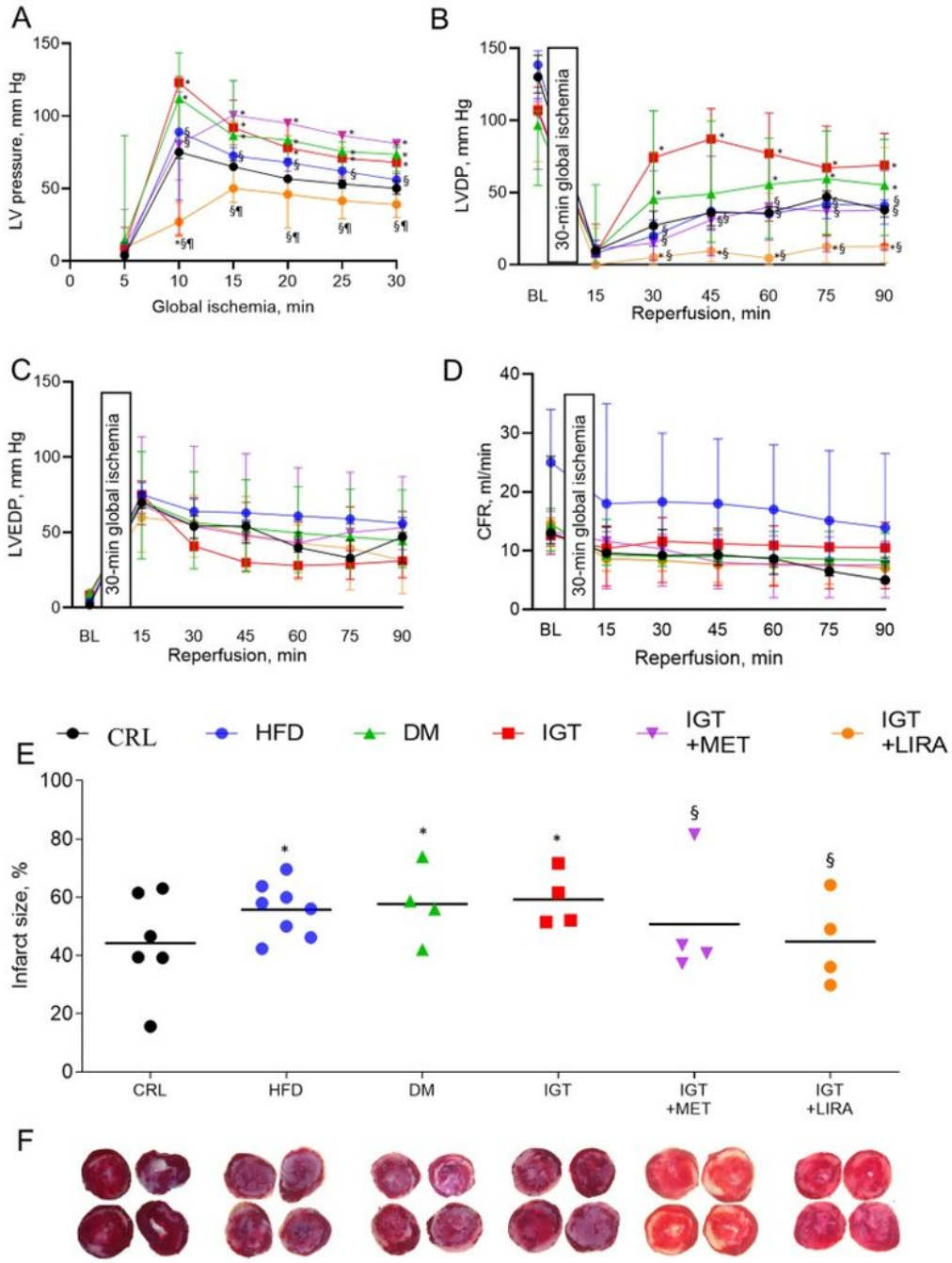

Figure 3

Functional parameters and myocardial infarct size in isolated rat hearts subjected to 30 min of global ischemia followed by 90 min of reperfusion. (A) Ischemic contracture, (B) LVDP, (C) LVEDP, (D) CFR values at baseline and during the experiment. Results are presented as median (25; 75) %. (E) Infarct size results are presented as dot plots with median values. (F) Representative images of heart slices stained

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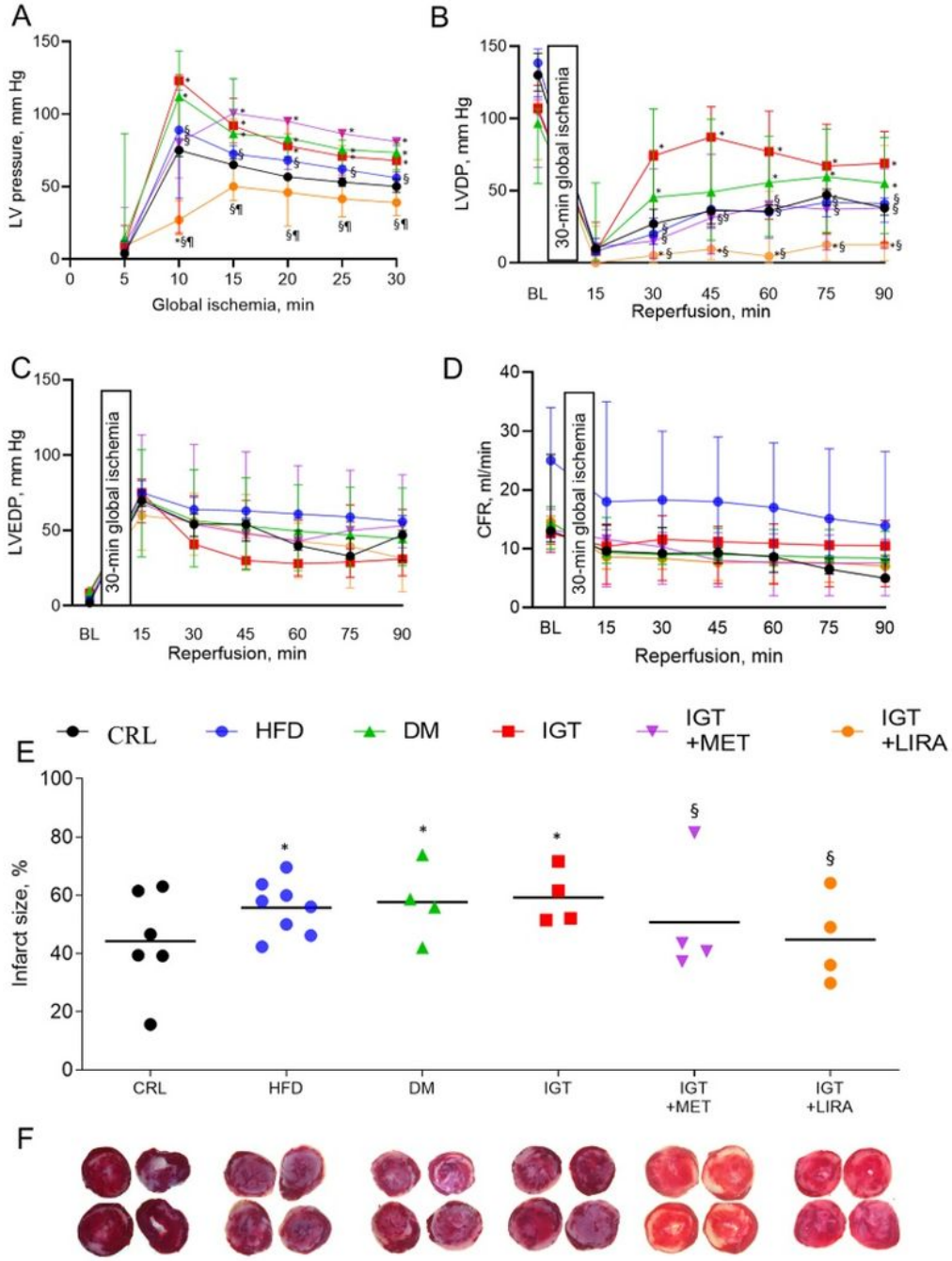

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