

Article

Hyperleptinemia results in systemic inflammation and the exacerbation of ischemia-reperfusion myocardial injury: the involvement of the JAK/STAT pathway

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Abstract: Hyperleptinemia potentiates the effects of many atherogenic factors, such as inflammation, platelet aggregation, migration, hypertrophy, proliferation of vascular smooth muscle cells, and endothelial cell dysfunction. The present study analysed the effects of long-term hyperleptinemia in an *in vivo* myocardial ischemia-reperfusion model to demonstrate whether the *in vivo* deleterious effect also affects cardiac structure and function. Rats were subcutaneously administered leptin for 8 days to estimate the involvement of the JAK/STAT pathway. Data from 58 male Wistar rats were included in the final analysis. Myocardial infarction (MI) was modelled by the 30-minute ligation of the main left coronary artery followed by 120-minute reperfusion. Hemodynamic measurements, electrocardiography monitoring, echocardiography, myocardial infarct size and area at risk, blood biochemical parameters, leptin, IL-6, TNF-alpha, FGF-21, and cardiomyocyte morphology were measured. Statistical analyses were performed using IBM SPSS Statistics v.26. Eight-day hyperleptinemia in rats led to increased blood pressure and heart rate, myocardial hypertrophy, impaired LV function, an increased frequency of ischemic arrhythmias, dyslipidaemia, systemic inflammation, and an increased size of induced myocardial infarction. The blockade of the JAK/STAT signalling pathway effectively reversed the negative effects of leptin, including increased blood pressure and total cholesterol.

Keywords: leptin; JAK/STAT pathway; myocardial infarction; hemodynamics; arrhythmias; dyslipidaemia; inflammation; cardiac remodeling

1. Introduction

Hyperleptinemia plays an important role in obesity-associated cardiovascular diseases, including atherosclerosis [1,2]. It is considered an important risk factor for coronary heart disease, particularly myocardial infarction (MI). Increases in the weight of white adipose tissue leads to leptin hypersecretion, as leptin is mainly produced by adipocytes [3]. The effects of hyperleptinemia are multidirectional, and evidence indicates that it may exaggerate ischemic injury to the myocardium. Therefore, the translation potential of experimental studies aimed at the modification of myocardial infarct size can be improved by using hyperleptinemia models. Theoretically, such an approach might be more relevant to clinical practice since many patients with acute coronary syndrome have an increased plasma leptin level.

There is a direct correlation between the amount of body fat and serum leptin concentration, reaching ≥ 200 ng/mL in morbidly obese individuals compared with 10 ng/mL in non-obese individuals [4]. The main physiological functions of leptin include an inhibition of food intake, a reduction of body weight, and the stimulation of energy expenditure [5]. Hyperleptinemia potentiates the effect of many atherogenic factors, including inflammation, platelet aggregation, migration, hypertrophy, proliferation of vascular smooth muscle cells, endothelial cell dysfunction, reactive oxygen species formation, and decreased paraoxonase activity. In addition, hyperleptinemia modulates the expression

of several vascular genes associated with atherosclerosis and abnormal angiogenesis, including cytokines, growth factors, and extracellular matrix proteins [6,7].

The expression of leptin receptors on the cardiomyocyte membrane has also been described, suggesting a direct effect of adipokines on myocardial function [2]. Several signalling pathways have been shown to be involved in cardiovascular regulation by leptin, including Janus activated kinase (JAK), signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein (MAP) kinase, and nitric oxide [5], which are associated with a direct hypertrophic effect and can induce cardiac remodelling [2,8]. Continuous short-term leptin infusion following MI in mice causes eccentric left ventricular dilation with increased systolic function [9]. In addition, the chronic blockade of leptin receptors (ObR) by the systemic administration of specific antibodies limits the development of post-infarct cardiac dysfunction in rats [10]. Interestingly, FGF21 expression is controlled by STAT3, a stress-responsive transcription factor that is also known to mediate leptin effects [11].

Cardiac ischemia-reperfusion injury (IRI) activates the renin-angiotensin-aldosterone system, resulting in the release of angiotensin II and endothelin-1, both of which drive myocardial remodelling via leptin induction and mediation [12]. Experimentally induced hyperleptinemia in rodents has been reported to result in hypertension and affect post-ischemic cardiac remodelling and myocardial dysfunction. However, short-term leptin administration reduces infarct size in isolated perfused rat hearts [13] and attenuates cardiomyocyte apoptosis after ischemia by increasing bcl-2 and survivin gene expression and by reducing caspase-3 activity [14]. Leptin has also been shown to possess cardioprotective properties in several *ex vivo* and *in vitro* studies performed in mice [15,16]. In contrast, clinical data suggest that the blood leptin levels are correlated with cardiovascular morbidity, obesity, MI, and heart failure [2,17,18,19]. Moreover, hyperleptinemia is often considered a surrogate marker of cardiovascular diseases [20]. Rats with acute MI demonstrate preservation of myocardial function when cardiac leptin activity is counteracted [21].

Therefore, the cardiovascular effects of leptin are complex, and the simultaneous influence of other factors can modify the experimental results. In this study, we addressed the hypothesis that long-term hyperleptinemia might lead to more extensive myocardial IRI in a rat model.

The aim of this study was to analyse the effects of long-term hyperleptinemia in an *in vivo* myocardial ischemia-reperfusion model to demonstrate whether the *in vivo* deleterious effect also affects cardiac structure and function by subcutaneously administering rats with leptin for a period of 8 days. As the heart is a high-energy-demand organ, we propose that a possible link between obesity and the development of cardiovascular diseases may be related to the effect of circulating leptin on inflammation, cholesterol, and glucose metabolism. The JAK/STAT signalling pathway has been implicated in the physiological and pathological effects of leptin [1], and we examined the JAK/STAT signalling pathway as a potential mechanism by which leptin influences IRI. For that purpose, we first analysed the effects of prolonged leptin administration on myocardial IRI, and then investigated whether the effects of leptin could be reversed by a specific inhibitor of the JAK/STAT pathway.

2. Results

2.1. Experiment 1

Chronic subcutaneous leptin administration (at a concentration of 0.33 $\mu\text{g}/\mu\text{l}$ and an infusion rate of 10 $\mu\text{l}/\text{hour}$ for 8 days, subcutaneously) altered hemodynamic and metabolic parameters, inflammatory markers, and myocardial IRI in a rat model of myocardial infarction.

In this experiment, the following four animal groups were analysed: the LEP group with chronic leptin administration was compared with the CON, SH, and PUMP+VEH groups.

On the day 8, the body weight increased significantly in all experimental groups compared with the baseline (Table 1).

Table 1. Body weight dynamics.

Group		Number	Initial body weight (g)	Day 8 body weight (g)	p
1	CON	8	293.0 (263.0; 295.0)	302.0 (270.5; 302.0)	0.010
2	SH	7	273.0 (262.0; 279.0)	281.0 (268.5; 288.5)	0.018
3	LEP	10	279.0 (270.0; 296.0)	267.0 (260.0; 281.0)	0.005
4	PUMP+VEH	8	291.0 (266.0; 295.5)	295.5 (274.5; 303.0)	0.017
5	JSI-124	10	289.0 (258.0; 297.0)	301.0 (267.0; 308.0)	0.017
6	DMSO	8	279.0 (270.5; 295.0)	288.0 (276.5; 303.5)	0.005
7	LEP+JSI-124	7	286.0 (278.0; 289.0)	275.0 (266.5; 281.5)	0.011
	p		0.787	0.046	

CON, control group; SH, sham surgery group; LEP, leptin administration group; PUMP+VEH, group with osmotic pump with vehicle; JSI-124, group with JSI-124 administration intraperitoneally; DMSO, group with vehicle administration intraperitoneally; LEP+JSI-124, simultaneous leptin and JSI-124 administration group. Values are expressed as medians and interquartile ranges. n=7-10 for each group.

There were no intergroup differences in body weight at the baseline or at the end of the experiment (Table 1).

Moreover, there were no differences between the CON, SH, LEP, and PUMP+VEH groups in terms of mean blood pressure and heart rate at the baseline, and between the groups CON, LEP and PUMP+VEH during ischemia and reperfusion (Table 2). Mean BP increased at the baseline in the LEP group compared with that in the CON group (p=0.003). In addition, the heart rate was higher in the LEP group than in the CON group at the end of ischemia (p=0.006).

Table 2. Systemic hemodynamics in all study groups.

Group		Number	Baseline	Ischemia 5 min	Ischemia 30 min	Reperfusion 60 min	Reperfusion 120 min	p
			1	2	3	4	5	
BP, mean (mmHg)								
1	CON	8	114.5 (108.5; 119.5)	69.0 (65.5; 71.0)	81.5 (78.0; 87.0)	58.0 (54.0; 64.0)	66.0 (60.5; 70.5)	<0.001 p ₁₋₂ =0.012 p ₁₋₃ =1.000 p ₁₋₄ <0.001 p ₁₋₅ =0.002

								<p>p₂₋₃=0.481</p> <p>p₂₋₄=1.000</p> <p>p₂₋₅=1.000</p> <p>p₃₋₄=0.016</p> <p>p₃₋₅=0.143</p> <p>p₄₋₅=1.000</p>
2	SH	7	119.0 (115.0; 122.0)	-	-	-	-	-
3	LEP	10	129.0 (127.0; 132.0)	69.5 (64.0; 76.5)	76.5 (71.0; 78.0)	60.0 (54.0; 62.0)	67.0 (61.0; 71.0)	<p><0.001</p> <p>p₁₋₂=0.030</p> <p>p₁₋₃=0.196</p> <p>p₁₋₄<0.001</p> <p>p₁₋₅=0.004</p> <p>p₂₋₃=1.000</p> <p>p₂₋₄=0.196</p> <p>p₂₋₅=1.000</p> <p>p₃₋₄=0.030</p> <p>p₃₋₅=1.000</p> <p>p₄₋₅=0.771</p>
4	PUMP+VEH	8	120.5 (117.5; 121.5)	71.0 (64.0; 76.5)	79.5 (75.5; 84.0)	54.0 (50.5; 60.5)	69.0 (64.5; 71.0)	<p><0.001</p> <p>p₁₋₂=0.034</p> <p>p₁₋₃=1.000</p> <p>p₁₋₄<0.001</p> <p>p₁₋₅=0.009</p> <p>p₂₋₃=1.000</p> <p>p₂₋₄=0.481</p> <p>p₂₋₅=1.000</p> <p>p₃₋₄=0.007</p> <p>p₃₋₅=0.690</p> <p>p₄₋₅=1.000</p>
5	JSI-124	10	107.0 (99.0; 125.0)	69.0 (67.0; 78.0)	72.5 (69.0; 81.0)	56.0 (54.0; 58.0)	67.0 (64.0; 71.0)	<p><0.001</p> <p>p₁₋₂=0.109</p> <p>p₁₋₃=0.237</p> <p>p₁₋₄<0.001</p> <p>p₁₋₅=0.001</p> <p>p₂₋₃=1.000</p> <p>p₂₋₄=0.030</p> <p>p₂₋₅=1.000</p> <p>p₃₋₄=0.011</p> <p>p₃₋₅=1.000</p> <p>p₄₋₅=0.897</p>
6	DMSO	8	118.5 (113.5; 122.0)	72.5 (65.0; 76.5)	76.0 (72.0; 80.5)	55.5 (50.5; 61.5)	68.5 (61.0; 72.5)	<p>0.001</p> <p>p₁₋₂=0.057</p>

								$p_{1-3}=0.820$ $p_{1-4}<0.001$ $p_{1-5}=0.007$ $p_{2-3}=1.000$ $p_{2-4}=0.481$ $p_{2-5}=1.000$ $p_{3-4}=0.027$ $p_{3-5}=0.969$ $p_{4-5}=1.000$
7	LEP+JSI-124	7	106.0 (103.0; 120.0)	79.0 (73.5; 79.0)	75.0 (72.5; 77.5)	58.0 (53.5; 60.0)	65.0 (60.5; 69.5)	<0.001 $p_{1-2}=1.000$ $p_{1-3}=0.225$ $p_{1-4}<0.001$ $p_{1-5}=0.003$ $p_{2-3}=1.000$ $p_{2-4}=0.041$ $p_{2-5}=0.346$ $p_{3-4}=0.346$ $p_{3-5}=1.000$ $p_{4-5}=1.000$
	p		0.001 $p_{1-3}=0.003$ $p_{3-5}=0.001$ $p_{3-7}=0.004$	0.2	0.021	0.816	0.993	
HR (beats/min)								
1	CON	8	379.2 (346.8; 388.3)	348.0 (342.0; 352.0)	310.0 (306.0; 324.0)	318.0 (196.5; 338.0)	307.0 (303.0; 314.5)	<0.001 $p_{1-2}=1.000$ $p_{1-3}=0.114$ $p_{1-4}=0.016$ $p_{1-5}=0.005$ $p_{2-3}=0.269$ $p_{2-4}=0.044$ $p_{2-5}=0.016$ $p_{3-4}=1.000$ $p_{3-5}=1.000$ $p_{4-5}=1.000$
2	SH	7	366.7 (364.5; 376.3)	-	-	-	-	-
3	LEP	10	371.5 (365.0; 389.7)	372.0 (355.0; 384.0)	360.0 (342.0; 384.0)	334.5 (324.0; 348.0)	302.0 (290.0; 308.0)	<0.001 $p_{1-2}=1.000$ $p_{1-3}=1.000$ $p_{1-4}=0.058$ $p_{1-5}<0.001$

								<p>p_{2,3}=1.000</p> <p>p_{2,4}=0.771</p> <p>p_{2,5}=0.003</p> <p>p_{3,4}=1.000</p> <p>p_{3,5}=0.024</p> <p>p_{4,5}=0.660</p>
4	PUMP+VEH	8	376.3 (364.3; 387.3)	348.0 (344.0; 360.0)	326.0 (306.0; 344.0)	349.0 (309.0; 357.0)	305.0 (290.54 313.0)	<p><0.001</p> <p>p_{1,2}=1.000</p> <p>p_{1,3}=0.020</p> <p>p_{1,4}=0.269</p> <p>p_{1,5}=0.001</p> <p>p_{2,3}=0.177</p> <p>p_{2,4}=1.000</p> <p>p_{2,5}=0.016</p> <p>p_{3,4}=1.000</p> <p>p_{3,5}=1.000</p> <p>p_{4,5}=0.969</p>
5	JSI-124	10	384.5 (384.0; 385.7)	378.0 (366.0; 402.0)	357.0 (340.0; 366.0)	311.5 (304.0; 322.0)	304.5 (290.0; 308.0)	<p><0.001</p> <p>p_{1,2}=1.000</p> <p>p_{1,3}=0.477</p> <p>p_{1,4}=0.001</p> <p>p_{1,5}<0.001</p> <p>p_{2,3}=1.000</p> <p>p_{2,4}=0.007</p> <p>p_{2,5}<0.001</p> <p>p_{3,4}=0.477</p> <p>p_{3,5}=0.047</p> <p>p_{4,5}=1.000</p>
6	DMSO	8	376.0 (371.3; 392.5)	344.0 (324.5; 361.0)	330.0 (322.0; 342.0)	318.0 (303.5; 320.0)	304.5 (289.0; 311.0)	<p><0.001</p> <p>p_{1,2}=0.690</p> <p>p_{1,3}=0.177</p> <p>p_{1,4}=0.001</p> <p>p_{1,5}<0.001</p> <p>p_{2,3}=1.000</p> <p>p_{2,4}=0.481</p> <p>p_{2,5}=0.044</p> <p>p_{3,4}=1.000</p> <p>p_{3,5}=0.219</p> <p>p_{4,5}=1.000</p>
7	LEP+JSI-124	7	389.7 (379.2; 396.5)	372.0 (366.0; 390.0)	378.0 (363.5; 390.0)	320.0 (312.0; 325.0)	297.0 (293.0; 306.0)	<p><0.001</p> <p>p_{1,2}=1.000</p> <p>p_{1,3}=1.000</p>

								<p>$p_{1-4}=0.041$</p> <p>$p_{1-5}=0.002$</p> <p>$p_{2-3}=1.000$</p> <p>$p_{2-4}=0.112$</p> <p>$p_{2-5}=0.007$</p> <p>$p_{3-4}=0.280$</p> <p>$p_{3-5}=0.023$</p> <p>$p_{4-5}=1.000$</p>
	p		0.18	0.005	<p><0.001</p> <p>$p_{1-3}=0.006$</p> <p>$p_{1-5}=0.008$</p> <p>$p_{1-7}<0.001$</p> <p>$p_{4-7}=0.008$</p>	0.069	0.702	

CON, control group; SH, sham surgery group; LEP, leptin administration group; PUMP+VEH, group with osmotic pump with vehicle; JSI-124, group with JSI-124 administration intraperitoneally; DMSO, group with vehicle administration intraperitoneally; LEP+JSI-124, simultaneous leptin and JSI-124 administration group. Values are expressed as medians and interquartile ranges. n=7-10 for each group.

Myocardial ischemia led to a significant decrease in mean BP in all groups compared to baseline ($p<0.05$). At the 60th minute and at the end of reperfusion, both the heart rate and mean BP decreased significantly in all seven experimental groups compared with the baseline. This reduction can be explained by cardiac dysfunction following acute myocardial infarction.

Cardiac morphology and function assessments were based on multiple echocardiographic parameters (Table 3).

Table 3. LV function in all groups on day 8 (echocardiographic data).

Group		Number	ED LVPW (mm)	ED IVS (mm)	LV EDD (mm)	LV ES (%)
1	CON	8	1.3 (1.2; 1.5)	1.0 (1.0; 1.1)	6.1 (6.1; 6.4)	67.3 (65.9; 68.9)
2	SH	7	1.5 (1.4; 1.6)	1.4 (1.3; 1.5)	6.4 (6.3; 6.5)	68.3 (66.4; 69.0)
3	LEP	10	2.1 (2.0; 2.3)	1.9 (1.9; 2.0)	7.2 (7.0; 7.6)	69.5 (66.1; 71.7)
4	PUMP+VEH	8	1.5 (1.3; 1.6)	1.4 (1.3; 1.5)	6.5 (6.2; 6.6)	70.9 (67.8; 72.0)
5	JSI-124	10	1.3 (1.1; .4)	0.9 (0.9; 0.9)	6.2 (6.0; 6.3)	70.5 (66.4; 71.4)
6	DMSO	8	1.3 (1.2; 1.4)	0.9 (0.9; 1.2)	6.4 (6.2; 6.5)	68.6 (66.3; 71.3)
7	LEP+JSI-124	7	2.1 (2.0; 2.2)	2.0 (1.9; 2.0)	7.2 (7.0; 7.3)	71.3 (69.7; 72.5)
			<p><0.001</p> <p>$p_{1-3}=0.001$</p> <p>$p_{3-5}=0.001$</p> <p>$p_{3-6}=0.001$</p> <p>$p_{4-7}=0.004$</p>	<p><0.001</p> <p>$p_{1-3}=0.001$</p> <p>$p_{3-5}<0.001$</p> <p>$p_{3-6}<0.001$</p> <p>$p_{4-7}=0.004$</p>	<p><0.001</p> <p>$p_{1-3}<0.001$</p> <p>$p_{3-5}<0.001$</p> <p>$p_{3-6}=0.014$</p> <p>$p_{4-7}=0.003$</p>	<p>0.020</p> <p>$p_{1-3}=0.041$</p>

			p ₅₋₇ =0.002 p ₆₋₇ =0.008	p ₅₋₇ <0.001 p ₆₋₇ =0.001	p ₅₋₇ <0.001	
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CON, control group; SH, sham surgery group; LEP, leptin administration group; PUMP+VEH, group with osmotic pump with vehicle; JSI-124, group with JSI-124 administration intraperitoneally; DMSO, group with vehicle administration intraperitoneally; LEP+JSI -124, simultaneous leptin and JSI-124 administration group; ED LVPW, end-diastolic left ventricular posterior wall thickness; ED IVS, end-diastolic interventricular septal thickness; LV EDD, left ventricular end-diastolic dimension; LV FS, left ventricular fractional shortening. Values are expressed as medians and interquartile ranges. n=7-10 for each group.

Echocardiography analyses revealed the development of hypertrophy, including dilation and a significant reduction in LV function, in leptin-treated animals versus CON, SH, and PUMP+VEH groups of rats (Table 3). Compared to the controls, hyperleptinemic rats had an increased end-diastolic left ventricular posterior wall thickness (p=0.001), end-diastolic interventricular septal thickness (p=0.001), and LV end-diastolic dimension (LV EDD) (p<0.001). These findings indicated significant LV enlargement, which markedly affected the LV systolic function. In particular, left ventricular fractional shortening (LV FS) was significantly reduced (p=0.041) in leptin-treated animals compared to CON. There were no differences in echocardiography data between groups SH and PUMP + VEH versus CON (Table 3). Thus, cardiac dysfunction, as measured by echocardiography on day 8, was exacerbated by leptin administration.

In addition, the number of ischemic arrhythmia episodes was analysed (Figure 2). In leptin-treated rats, there was a significantly higher number of ischemic arrhythmias than the CON and PUMP + VEH groups (Figure 2; p=0.034 and p=0.019, respectively).

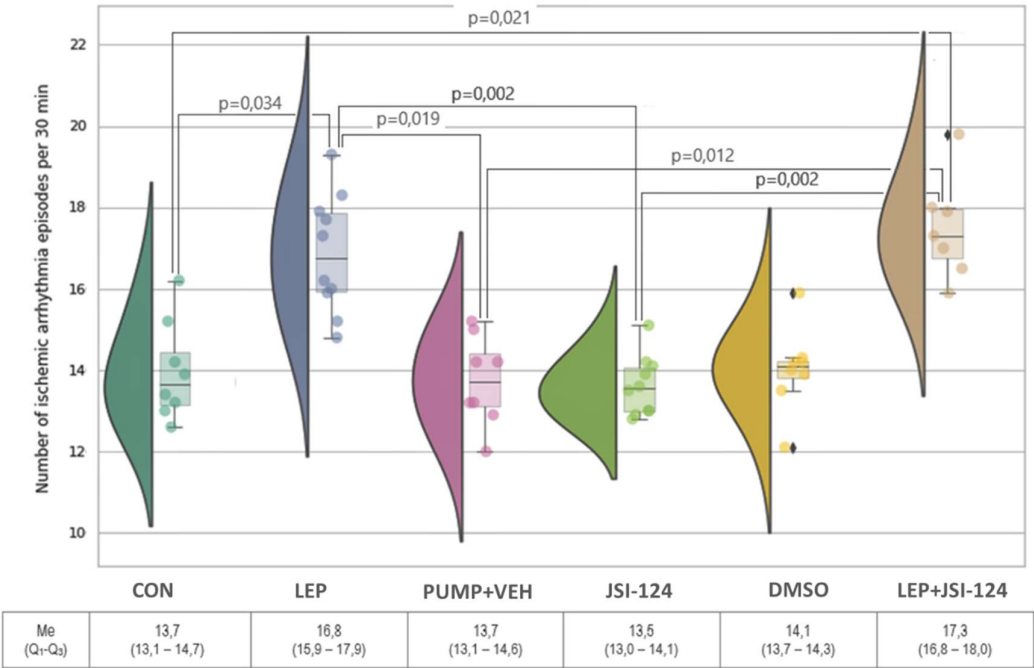


Figure 2. Number of arrhythmia episodes per 30 min registered on day 8 during ischemia in all groups.

The validity of the model of chronic hyperleptinemia has been confirmed by significantly elevated plasma leptin levels in the LEP group compared to the CON group. Compared with CON, leptin-treated rats developed higher plasma levels of total cholesterol and LDL (Table 4), as well as troponin-I, IL-6, TNF-alpha, and lower plasma levels of FGF-21 (Table 5). However, the levels of blood glucose, HDL, and plasma triglycerides were not significantly different between the LEP and CON groups. There were no differences in blood plasma parameters between the SH and PUMP+VEH groups compared with the CON group, except for the SH group with respect to troponin-I (Table 4 and 5).

Table 4. Biochemical parameters of blood plasma in all study groups.

Group		Number	Glucose (mmol/l)	Total cholesterol (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	Triglyceride (mmol/l)
1	CON	8	4.7 (4.5; 5.0)	4.3 (4.1; 4.5)	1.3 (1.2; 1.4)	2.5 (2.3; 2.9)	1.2 (1.1; 1.3)
2	SH	7	5.0 (4.6; 5.3)	4.4 (4.0; 4.6)	1.4 (1.3; 1.7)	3.0 (2.3; 3.1)	1.2 (1.1; 1.2)
3	LEP	10	4.9 (4.7; 5.1)	5.6 (5.1; 5.9)	1.4 (1.1; 1.8)	3.5 (3.3; 3.6)	1.1 (1.1; 1.3)
4	PUMP+VEH	8	4.7 (4.4; 5.2)	4.5 (4.2; 4.8)	1.7 (1.5; 1.9)	2.6 (2.3; 2.9)	1.2 (1.2; 1.3)
5	JSI-124	10	4.9 (4.8; 5.2)	4.3 (4.1; 4.7)	1.5 (1.4; 1.7)	2.5 (2.1; 2.9)	0.3 (0.3; 0.4)
6	DMSO	8	4.6 (4.4; 5.2)	4.5 (4.0; 4.8)	1.7 (1.3; 1.8)	2.6 (2.3; 3.0)	1.3 (1.2; 1.3)
7	LEP+JSI-124	7	5.1 (5.0; 5.5)	4.7 (4.3; 4.7)	1.4 (1.4; 1.5)	3.2 (2.9; 3.0)	0.9 (0.4; 1.0)
			0.406	<0.001 p1-3=0.003 p2-3=0.005 p3-4=0.043 p3-5=0.001 p3-6=0.008 p3-7=0.028	0.140	<0.001 p1-3=0.008 p3-4=0.012 p3-5<0.001 p3-6=0.027	<0.001 p1-5=0.002 p1-7=0.040 p2-5=0.035 p3-5=0.007 p4-5<0.001 p4-7=0.014 p5-6<0.001 p6-7=0.010

CON, control group; SH, sham surgery group; LEP, leptin administration group; PUMP+VEH, group with osmotic pump with vehicle; JSI-124 – group with JSI-124 administration intraperitoneally; DMSO – group with vehicle administration intraperitoneally; LEP+JSI-124 – simultaneous leptin and JSI-124 administration group. Values are expressed as medians and interquartile ranges. n=7-10 for each group

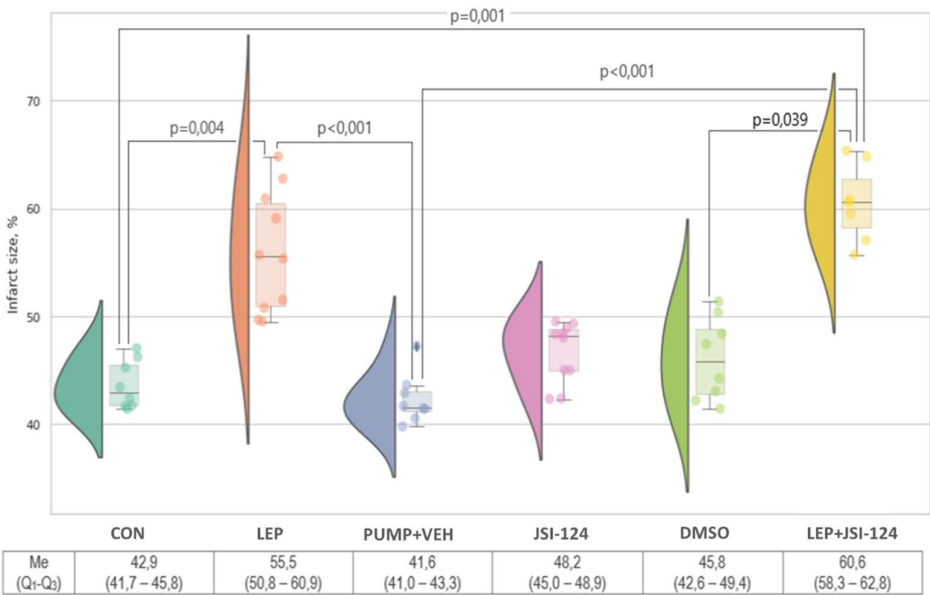
Table 5. Plasma troponin-I, adipokines and cytokines in all study groups

Group		Number	Troponin-I (ng/mL)	Leptin (ng/mL)	FGF-21 (pg/mL)	IL-6 (pg/mL)	TNF-alpha (pg/mL)
1	CON	8	42.0 (39.0; 47.0)	3.2 (3.0; 3.8)	20.1 (15.0; 31.5)	9.8 (9.5; 11.8)	68.8 (61.2; 73.6)
2	SH	7	2.1 (1.5; 3.1)	3.5 (3.2; 3.7)	32.4 (24.6; 35.7)	13.9 (9.6; 15.9)	64.8 (54.9; 73.1)
3	LEP	10	84.5 (79.0; 87.0)	70.2 (65.0; 71.6)	6.6 (3.3; 7.8)	24.9 (20.8;29.9)	182.0 (164.7; 234.9)

4	PUMP+VEH	8	43.0 (40.0; 47.5)	3.5 (3.1; 3.9)	26.3 (16.3; 28.9)	10.2 (8.6; 12.0)	94.0 (53.6; 102.1)
5	JSI-124	10	45.0 (38.0; 47.0)	2.8 (2.6; 3.2)	33.7 (22.0; 40.5)	11.1 (10.6; 14.5)	153.0 (75.7; 168.7)
6	DMSO	8	41.0 (38.5; 43.5)	2.9 (2.7; 3.2)	27.8 (25.5; 30.1)	11.8 (10.4; 13.7)	80.6 (71.2; 86.7)
7	LEP+JSI-124	7	77.0 (72.5; 81.0)	73.1 (69.1; 76.1)	3.3 (2.1; 5.7)	16.7 (15.1; 17.5)	80.6 (71.2; 86.7)
	p		<0.001 p _{1,3} =0.002 p _{1,4} =0.004 p _{1,5} =0.003 p _{1,6} <0.001 p _{6,7} =0.015 p _{1,2} <0.001 p _{2,3} <0.001 p _{2,4} <0.001 p _{2,5} <0.001 p _{2,6} <0.001 p _{2,7} <0.001	<0.001 p _{1,3} =0.032 p _{1,7} =0.021 p _{1,5} <0.001 p _{1,6} =0.001 p _{5,7} <0.001 p _{6,7} =0.001	<0.001 p _{2,3} =0.003 p _{2,7} =0.003 p _{1,4} =0.049 p _{1,5} <0.001 p _{1,6} =0.011 p _{1,7} =0.048 p _{5,7} =0.001 p _{6,7} =0.012	<0.001 p _{1,3} <0.001 p _{2,3} =0.023 p _{1,4} <0.001 p _{1,5} =0.003 p _{1,6} =0.011	<0.001 p _{1,3} =0.001 p _{1,7} =0.004 p _{2,3} =0.001 p _{2,7} =0.004 p _{3,6} =0.037

CON, control group; SH, sham surgery group; LEP, leptin administration group; PUMP+VEH, group with osmotic pump with vehicle; JSI-124 – group with JSI-124 administration intraperitoneally; DMSO – group with vehicle administration intraperitoneally; LEP+JSI-124 – simultaneous leptin and JSI-124 administration group. Values are expressed as medians and interquartile ranges. n=7-10 for each group.

The myocardial infarct size and the area at risk are shown in Figure 3. The infarct size in rats with hyperleptinemia was larger than that in rats from the CON group (p=0.004) and LEP group (p<0.001) (Figure 3A). There were no intergroup differences in the area at risk (p>0.05) (Figure 3B).



(A)

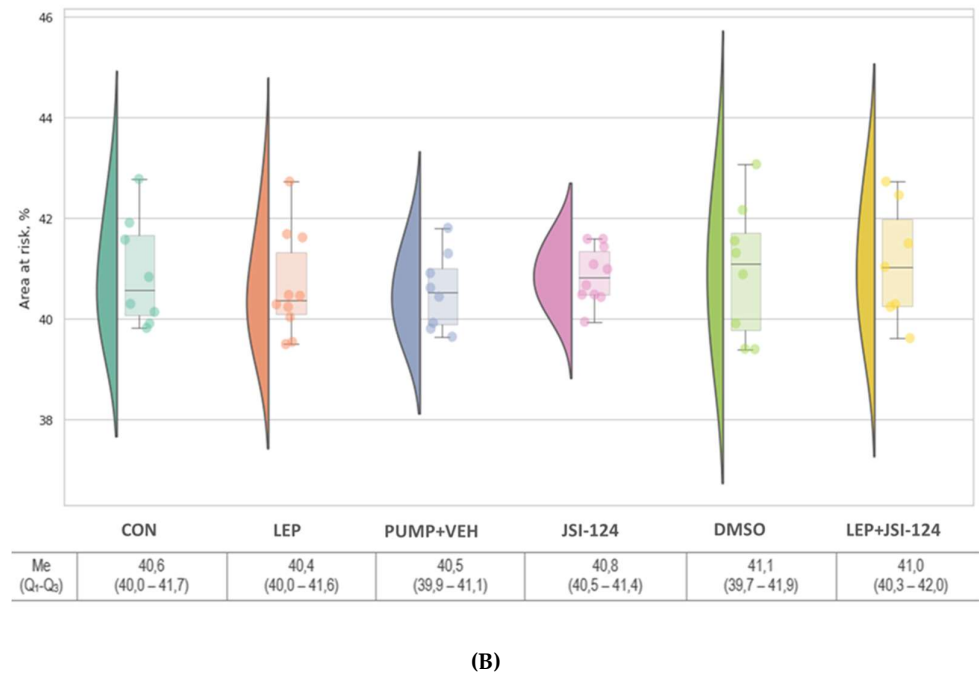


Figure 3. Infarct size (A) expressed as a percentage of the area at risk and area at risk (B) expressed as a percentage of the left ventricle after 30 min of ischemia followed by 120 min of reperfusion in each group. Values are median and interquartile range. n=7-10 for each group.

2.2. Experiment 2

Long-term JAK/STAT inhibition (at a dose of 1 mg/kg/d for 7 days intraperitoneally) had no effect on hemodynamic and metabolic parameters, inflammatory markers, or myocardial IRI in rats with myocardial infarction.

In this experiment, the following four animal groups were analysed: JSI-124 group with long-term intraperitoneal infusion of JSI-124 (JAK/STAT inhibitor) was compared to the CON, SH, and DMSO groups.

There were no differences between the CON, SH, JSI-124, and DMSO groups in terms of the mean blood pressure and heart rate at the baseline, and during ischemia and reperfusion in the groups CON, JSI-124 and DMSO (Table 2). However, in the JSI-124 treated group, the heart rate was higher than in the CON group at the end of ischemia (p=0.008).

JAK/STAT inhibition did not influence the echocardiography data of rats. The parameters did not differ between the study groups (Table 3).

In Experiment 2, we found that the administration of JSI-124 did not significantly change the frequency of ischemic arrhythmias when compared to the DMSO (p>0.05, Figure 2).

JSI-124 administration was not associated with changes in metabolic parameters (Table 4 and 5); the only exception was a significant decrease in plasma triglyceride levels compared to the CON group (p=0.002) (Table 4).

The infarct size (Figure 3A) and area at risk (Figure 3B) in rats administered JSI-124 were not significantly different from those in the other groups (p>0.05).

2.3. Experiment 3

Inhibition of the JAK/STAT pathway partially reverses the changes induced by leptin administration in rats. In this experiment, the following three animal groups were analysed: LEP, JSI-124, and LEP+JSI-124.

Our results showed that the hemodynamic, metabolic, inflammation, and ischemic myocardial conditioning were impaired after chronic subcutaneous leptin administration. Next, we examined whether the administration of a JAK/STAT inhibitor, JSI-124, could rescue the impairments induced by leptin treatment. Because the JAK/STAT signalling pathway has been reported to participate in a number of metabolic and cardiovascular functions [25], JSI-124 without leptin application also influences heart conditioning (Experiment 2). Based on the results of Experiment 2 with JSI-124, we conducted an experiment with the application of leptin and JSI-124 (Figure 1) to determine whether blocking the JAK/STAT signalling pathway can rescue the leptin-induced impairments of metabolic and heart conditioning. This possibility was evaluated by conducting and comparing the experiments after subcutaneous leptin administration, after intraperitoneal infusion of JSI-124, and after both leptin and JSI-124 administration (Experiment 3).

No differences were observed between LEP, JSI-124, and LEP+JSI-124 in terms of body weight at the baseline and at the end of the experiment (Table 1).

Similarly, there were no differences in the heart rate during ischemia and reperfusion between the groups (Table 2). However, JAK/STAT inhibition led to a decrease in the baseline mean blood pressure in the group treated only with JSI-124 compared with leptin administration ($p=0.001$) in the group with simultaneous leptin and JSI-124 administration compared with the LEP group ($p=0.004$).

There were no intergroup LEP and LEP+JSI-124 differences in the incidence of ischemic arrhythmia (Figure 2). However, JAK/STAT blockade without leptin administration led to a decrease in the incidence of ischemic arrhythmias compared with leptin and JSI-124 rats treated with leptin alone (Figure 2).

In the LEP and LEP+JSI-124 groups, ED LVPW, ED IVS, and LV EDD were more deteriorated than in animals receiving JSI-124 alone, according to ECHO data (Table 3).

Blood plasma analysis revealed several significant differences. JSI-124 administration compared with rats treated with leptin and leptin and JSI-124 was accompanied by a significant reduction in total cholesterol (Table 4) and leptin (Table 5). Differences were found between the LEP and JSI-124 groups. Smaller concentrations of LDL and triglycerides (Table 4) and troponin-I and IL-6 (Table 5) were observed in the JSI-124 group. Animals in which the JAK/STAT signalling pathway was blocked had an increased FGF-21 plasma concentration compared with the LEP and LEP+JSI-124 groups (Table 5).

The infarct size and area at risk among rats from the LEP, JSI-124, and LEP+JSI-124 groups did not differ ($p>0.05$) (Figure 3A, 3B).

Together, these results suggest that blocking the JAK/STAT pathway compared with leptin effects is sufficient to restore hemodynamic, metabolic, and inflammation, but not myocardial impairment.

2.4. Post-regression analysis

A post-regression analysis of the role of the studied indicators on myocardial infarct size and the area at risk was performed. As a result, the correlation between infarct size and the level of leptin was found to be high ($r=0.830$, Cheddock scale) and statistically significant ($p<0.001$). In the resulting model, the leptin level determined the size of the infarction by 68.9% ($R^2=0.689$).

The observed dependence is described by the following equation:

$$Y_{\text{infarct size}} = 44.281 + 0.193 \cdot X_{\text{leptin}}$$

where $Y_{\text{infarct size}}$ is the infarction size (%) and X_{leptin} is the blood plasma leptin level (ng/ml). According to this equation, if the leptin level increased by 1 ng/ml, an increase of 0.193% in infarct size would be expected.

The Darbina-Watson criterion was 1.94. As this was in the range of 1 to 3, the independence of observations was respected. Estimation of the normal distribution of residues using the Shapiro-Wilk criterion ($p < 0.001$) indicated a normal distribution of residues, confirming the application of the regression model.

This pattern was found only for plasma leptin concentrations in relation to infarct size.

2.5. Effects of JSI-124 on attenuation of cardiomyocytes hypertrophy in animals receiving leptin

The size of the heart apex cardiomyocytes was assessed using light microscopy in the field of view (Figure 4). Among the four different groups (CON, LEP, JSI-124, and LEP+JSI-124), no significant intergroup difference was observed for cell diameter: $12.3 \pm 0.1 \mu\text{m}$, $13.7 \pm 0.2 \mu\text{m}$, $12.7 \pm 0.2 \mu\text{m}$, and $13.1 \pm 0.2 \mu\text{m}$ in 1, 3, 5, and 7 groups ($p > 0.05$). However, the cell sectional area of cardiomyocytes in rats from the LEP group ($324.7 \pm 36.7 \mu\text{m}^2$) and the LEP+JSI-124 group ($336.2 \pm 38.3 \mu\text{m}^2$) was significantly larger than that in the CON group ($262 \pm 33.8 \mu\text{m}^2$) ($p < 0.01$) and significantly larger than that in the JSI-124 group ($277 \pm 38.1 \mu\text{m}^2$) ($p < 0.01$). The cross-sectional area of cardiomyocytes was not different between the JSI-124 ($277 \pm 38.1 \mu\text{m}^2$) and CON groups ($p > 0.05$).

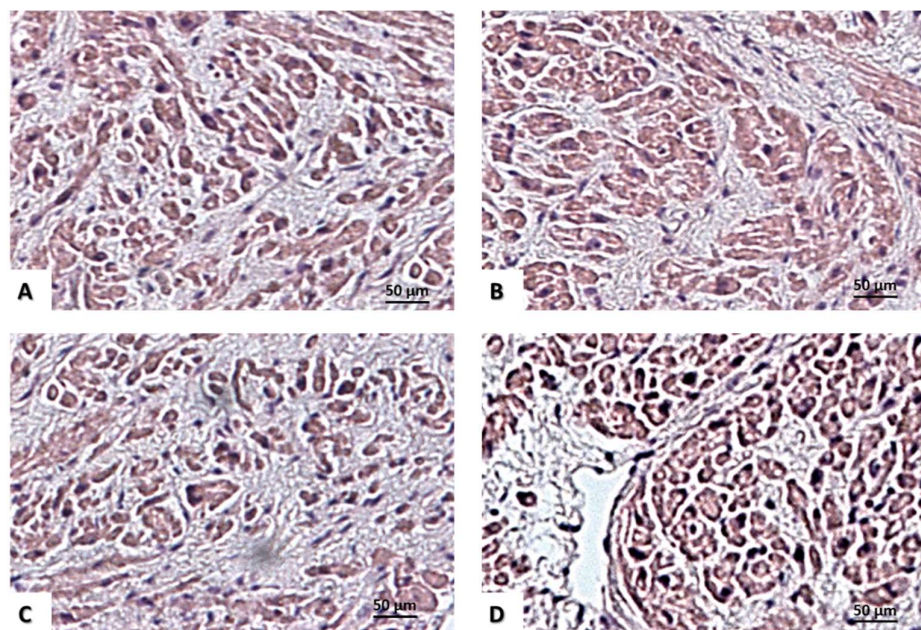


Figure 4. Myocardium staining with haematoxylin-eosin. Scale bar, 50 μm ($\times 400$). (A) Control group; (B) group with chronic leptin administration; (C) group with chronic administration of JAK/STAT inhibitor – JSI-124 (Cucurbitacin-I); (D) group with chronic administration of leptin and JSI-124.

3. Discussion

This study provides evidence that the long-term elevation of plasma leptin levels is associated with significant changes in hemodynamic and metabolic parameters, the induction of systemic inflammation, myocardial hypertrophy, and increased myocardial

IRI. In addition, the pharmacological inhibition of the JAK/STAT3 pathway by the intraperitoneal injection of JSI-124 was found to reverse leptin-induced changes in certain hemodynamic and metabolic parameters, as well as inflammatory markers. These findings suggest that leptin-JAK/STAT3 signalling could be involved in mediating leptin-associated mechanisms of both metabolic dysfunction and ischemic arrhythmogenesis in rats. Previous studies have provided insights regarding the mechanisms by which leptin-STAT signalling is implicated in the development of myocardial injury [1,2,8]. In addition to the leptin-STAT signalling pathway, other pathways, such as the MAPK and PI3K/AKT signalling pathways, are involved in the effector mechanisms of inflammatory responses [1]. Further study will be needed to determine the involvement of different signalling pathways in leptin-induced alterations and the interactions between them.

Previous reports have suggested that leptin plays a complex and variable role in the regulation of hemodynamic, metabolic, and inflammatory conditions and ischemic/reperfusion myocardial damage, and can produce opposite metabolic effects under different conditions or contexts [10,15,26]. On the one hand, leptin may exert cardioprotective effects [27] when administered acutely. On the other hand, evidence suggests that continuously elevated levels of leptin may be associated with maladaptive effects through hemodynamic factors, such as increased heart rate and blood pressure [28,29], metabolic changes including augmented fatty acid or impairment of glucose utilisation [30,31], induced cardiac apoptosis [32], or structural cardiac changes, such as cardiac lipid accumulation [31,33], and increased myocardial hypertrophy [34]. Obesity-related hyperleptinemia is accompanied by a low-grade inflammatory profile, characterised by increased circulating levels of IL-6, IL-12, and TNF- α , and reduced concentrations of IL-10. Whether hyperleptinemia is a cause or consequence of the systemic inflammatory milieu in humans is worthy of further consideration in clinical studies and research [30]. Furthermore, our rat model may also simulate clinical scenarios of patients who experience acute myocardial infarction and reperfusion coinciding with leptin overexpression associated with a pre-existing inflammatory state. Adverse consequences attributed to this unfavourable association have been observed in patients suffering from inflammatory bowel disease [35] or rheumatoid arthritis [36], who exhibit a disproportionally higher degree of post-MI heart failure. Therefore, severe infection may render patients vulnerable to serious cardiovascular complications and progression of heart failure [37]. Our results imply that a worse outcome of post-MI cardiac dysfunction may be attributed to excessive blood leptin concentration.

In the present study, rats treated with leptin for 8 days developed structural and metabolic alterations, including cardiac hypertrophy, dyslipidaemia, a more pronounced inflammatory response with IL-6 and TNF- α elevation, and FGF-21 plasma level depression. Although leptin administration in the current study increased the serum leptin levels, there was no significant difference in body weight, which is consistent with an earlier study [38]. The characteristics and metabolic parameters at the end of the study are consistent with a previous report, indicating that this animal model mimics the classical insulin resistance and leptin resistance features of human obesity [39]. This may explain the increase in mean BP and heart rate in the leptin-treated group.

Furthermore, there is evidence for the induction of both murine and human cardiomyocyte hypertrophy in response to leptin treatment, which appears to be mediated by the activation of extracellular signal-regulated kinase 1/2 and phosphatidylinositol-3 kinase [40]. Although some *in vitro* results suggest that leptin contributes to adverse cardiac remodelling and hypertrophy, the results from *in vivo* animal and human studies are inconclusive with regards to the direct role of leptin in cardiac hypertrophy [41].

In our study, we found myocardial hypertrophy and an increase in the cell cross-sectional area of cardiomyocytes in rats that received leptin. This pro-hypertrophic effect

of leptin was not abolished by JSI-124, indicating that it is not dependent on the JAK/STAT pathway. Additionally, we found that elevated blood leptin levels drove excessive myocardial remodelling, which may lead to the progression of heart failure. As a consequence of structural changes in the myocardium and pro-arrhythmogenic effects, the administration of leptin was also accompanied by LV end-diastolic dimension elevation and a reduction of markers, such as LV systolic function and left ventricular fractional shortening.

Autocrine leptin signalling plays an important role in cardiomyocyte metabolism [2]. This may explain the more pronounced ischemic myocardial damage in rats receiving leptin. Hyperleptinemic rats had a larger infarct size and higher troponin-I concentration compared to the controls. A previous study showed that hearts from obese Zucker rats exhibited larger infarct size following ischemia–reperfusion than the control rats [42]. Our results confirmed the findings of an earlier study demonstrating that high leptin levels, which were observed in fat-fed hypertensive rats compared to hypertensive-glucose intolerant rats (with lower leptin levels), and hyperleptinemia resulted in larger myocardial infarct size [43]. More importantly, we found that JSI-124 preconditioning did not reduce myocardial damage in leptin-treated rats, possibly due to the small dose of JSI-124. Similarly, the long-term inhibition of the JAK/STAT pathway (at a JSI-124 dose of 1 mg/kg/d) did not affect hemodynamics, metabolism (except triglycerides), inflammation, or ischemic myocardial injury in rats with myocardial infarction model compared with the controls.

The upregulation of leptin and the associated elevation of TNF-alpha, IL-6, and atherogenic lipoproteins are known to be involved in the pathological mechanisms of many human diseases, including cardiovascular, inflammatory, and autoimmune diseases, as well as inflammation-associated cancers [33,44]. Blocking leptin signalling is effective in treating experimental models of these diseases [45–47]. Recently, the involvement of leptin in the mechanisms of ischemic heart disease has also been demonstrated [48,49]. For example, a multicentre retrospective study suggested that blood leptin concentration is a significant risk factor for coronary heart disease in obese subjects [50]. In our study, we artificially elevated leptin levels to reproduce the pathological states caused by inflammatory and dysmetabolic conditions (Figure 5).

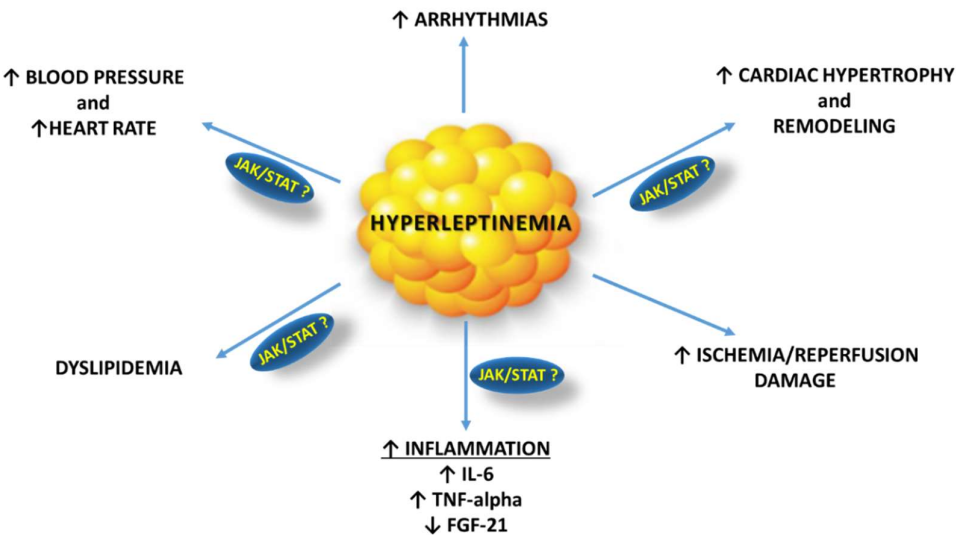


Figure 5. Potential mechanisms by which hyperleptinemia influences cardiac function, and potential association with the JAK/STAT pathway according to the results of our experiment. Hyperleptinemia may exert maladaptive effects through hemodynamic factors, such as increased heart rate

and blood pressure, metabolic and inflammatory changes, including dyslipidaemia, reduced cardiac output, or structural cardiac changes, such as myocardial hypertrophy.

4. Materials and Methods

4.1. Animals

This study was conducted in conformance with the policies and procedures detailed by the “Institutional Animal Care and Use Committee” (IACUC). All animal experimental protocols and procedures were reviewed and approved by the Committee for the Control of the Maintenance and Use of Laboratory Animals of the V. A. Almazov National Medical Research Center (date: 06-FEB-2020, application number: 20-01).

All experiments involved male Wistar rats aged 11–12 weeks and weighing 250–300 g (Pushchino, Moscow region, Russian Federation). Body weight was assessed prior to all procedures and on day 8 of the experiment, before myocardial infarction modelling. The rats were housed under standard conditions in a humidity-controlled (50–60%) and temperature-controlled (22–24°C) facility on a 12-h light-dark cycle (lights on at 8:00 AM). All rats were acclimatised for 2 weeks before the experiments with *ad libitum* access to food and water. All surgeries were performed under anaesthesia, and all efforts were made to minimise suffering.

Sixty-two male Wistar rats were used in the experiment. Four rats were removed from the study early: two died from anaesthetic failures and two died after myocardial infarction initiation. Thus, data from 58 rats were included in the final analysis.

4.2. Drugs

Recombinant rat leptin (endotoxin level <0.10 EU per 1 µg of protein by the limulus amoebocyte lysate method) (R&D Systems, Minneapolis, MN, USA) was reconstituted at 1 mg/mL in a sterile vehicle: 20 mM Tris-HCl, pH 8.0. Chronic leptin treatment was delivered by an osmotic mini-pump (model 2ML1; ALZET, Cupertino, CA, USA) with an initial leptin concentration of 0.33 µg/µl and an infusion rate of 10 µl/hour for 8 days, according to a previously described protocol [22].

JSI-124 (cucurbitacin I), a specific inhibitor of JAK/STAT, was purchased from Sigma-Aldrich (St. Louis, MO, USA). A 1 mg/ml JSI-124 stock solution was prepared in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA), stored at –20°C, and diluted in 0.9% NaCl to a concentration of 150 mM. JSI-124 was administered intraperitoneally at a daily dose of 1 mg/kg for 7 days, as described in an earlier study [23].

4.3. Osmotic pump implantation

An osmotic mini-pump was implanted subcutaneously in the midscapular region under isoflurane anaesthesia (Butler Schein Animal Health, Dublin, OH, USA) via an aesthetic vaporizer (Ohmeda; BOC Health Care, Steeton, UK). Continuous infusion of leptin was initiated for 8 days. After implantation, the animals were housed individually in normal cages under standard conditions.

4.4. Hemodynamic measurements and electrocardiography (ECG) monitoring

Heart rate, arterial blood pressure measurements, and ECG registration were performed during the experiment on day 8 after pump implantation, summarized in the following stages: initiation of anaesthesia, 1 h before ischemia, and coronary artery ligation up until the end of the experiment. After a midline cervical incision, the right carotid artery was cannulated for continuous blood pressure measurements. Heart rate and arterial blood pressure signals were recorded using a computer-based PhysExp Mini system (Cardioprotect Ltd., St. Petersburg, Russia).

A 3-lead surface ECG was recorded from subcutaneous needle electrodes attached to each limb in the prone position using the INCART acquisition and analysis system (LLC

Ltd “INCART”, Saint-Petersburg, Russia). Arrhythmias were quantified according to Lambeth Convention guidelines [24]. Differences in the number of ischemic arrhythmia episodes per animal were analysed.

4.5. Echocardiography

Echocardiography (ECHO) was performed on day 8 of the experiment, before myocardial IRI modelling, under light isoflurane anaesthesia using 1.5% isoflurane (Isoflurane Baxter, Baxter A/S Allerød, Denmark) applied through a face mask. Cardiac morphology and function were analysed with ECG-gating at a heart rate of 400–450 bpm using a commercially available system (Vevo 2100; Fujifilm VisualSonics, Toronto, Canada).

ECG was monitored to verify that ECHO examinations were performed at a heart rate of 400–450 bpm. The ECHO parameters were measured using M-mode tracings for parasternal long- and short-axis views. All ECHO examinations were performed by an experienced technician who was blinded to the treatment groups and assessed by a blinded investigator. Each measurement was performed on at least three cardiac cycles, and the average values were calculated. The study parameters included end-diastolic left ventricular posterior wall thickness, end-diastolic interventricular septal thickness, left ventricular end-diastolic diameter, and left ventricular fractional shortening.

4.6. Surgical preparation and myocardial infarction (MI) modeling

The rats were initially anaesthetized in a transparent acrylic box flushed with 2.5–3.0% isoflurane. After the induction of anaesthesia, which lasted from 5 to 10 min, the rats were removed from the box. To manage 1.5% isoflurane anaesthesia and minimise respiratory complications, the rats were carefully intubated orotracheally and mechanically ventilated with oxygen-enriched air (FiO₂ 35%) after tracheal intubation. Anaesthesia was maintained until the end of the experiment and the animals were euthanized. The respiratory rate was adjusted to maintain the arterial pCO₂ within physiological limits. A thermistor probe (8.0 mm) was inserted into the rectum to monitor the core body temperature that was maintained at normothermia between 37.0°C and 38.0°C. Body temperature was maintained using a feedback-controlled heating pad (TCAT2LV controller; Physitemp Instruments Inc., Clifton, NJ, USA).

After a 30-minute stabilisation period, left lateral thoracotomy and pericardiotomy were performed, and a 6-0 silk thread was passed below the main left coronary artery. The ends of the thread were passed through a propylene tube to form a snare. Successful coronary occlusion and MI initiation were verified visually by epicardial cyanosis and ST-segment elevation on ECG. Myocardial IRI was induced by a 30-min occlusion of the left coronary artery followed by 120-min of reperfusion. Reperfusion was verified by ST-segment depression on ECG and the appearance of local hyperaemia in the left ventricular area, which was previously ischemic and pale. At the end of the reperfusion, Evans blue was injected intra-aortically, and the rats were euthanized by isoflurane overdose.

4.7. Terminal sampling

At the end of the protocol (on day 8), blood samples from the aorta (4 mL) were collected in tubes containing EDTA and centrifuged at 3,000 × g for 15 min at ambient temperature. The resulting supernatants were collected and stored at –80°C until use. The heart apex was removed and fixed in 10% buffered neutral formaldehyde (pH 7.4) for 1 d and processed using routine histological examination. The hearts were then processed for the AR and IS measurements.

4.8. Infarct size measurement and area at risk

Excised hearts were cut into 2-mm thick transverse slices. The slices were incubated at 37°C for 20 min in 1% 2,3,5-triphenyl tetrazolium chloride buffer. The slices were then

placed in 10% formaldehyde for 10 min to increase the contrast between stained tissue with a deep red colour and non-stained tissue. Slices were then photographed, and the percentages of the area at risk (AR) and infarct size (IS) were calculated using an image analysis program (ImageJ) bundled with 64-bit Java 1.8.0_172). AR was expressed as a percentage of the whole slice, and IS was expressed as a percentage of AR. The values of AR and IS for each heart were obtained by summarising the data of the slices and calculating the mean values.

4.9. The experimental protocol and design

The animals were randomly (random number generation) assigned to one of seven study groups shown in Figure 1:

- (1) Control group (CON), n=8: control MI modelling according to the protocol described above.
- (2) Sham surgery operated animals group (SH), n=7: Thoracotomy was performed without coronary ligation.
- (3) Group with chronic administration of leptin using an osmotic mini-pump (LEP), n=10.
- (4) Leptin-control group with chronic administration of vehicle (20 mM Tris-HCl, pH 8.0) using an osmotic mini-pump (PUMP+VEH), n=8.
- (5) Group with chronic intraperitoneal administration of JAK/STAT inhibitor JSI-124 (Cucurbitacin-I) (JSI-124), n=10.
- (6) JSI-124-control group with chronic intraperitoneal administration of vehicle (DMSO + NaCl) (DMSO), n=8.
- (7) Group with chronic administration of leptin using the osmotic mini-pump and JSI-124 intraperitoneal administration (LEP+JSI-124), n=7.

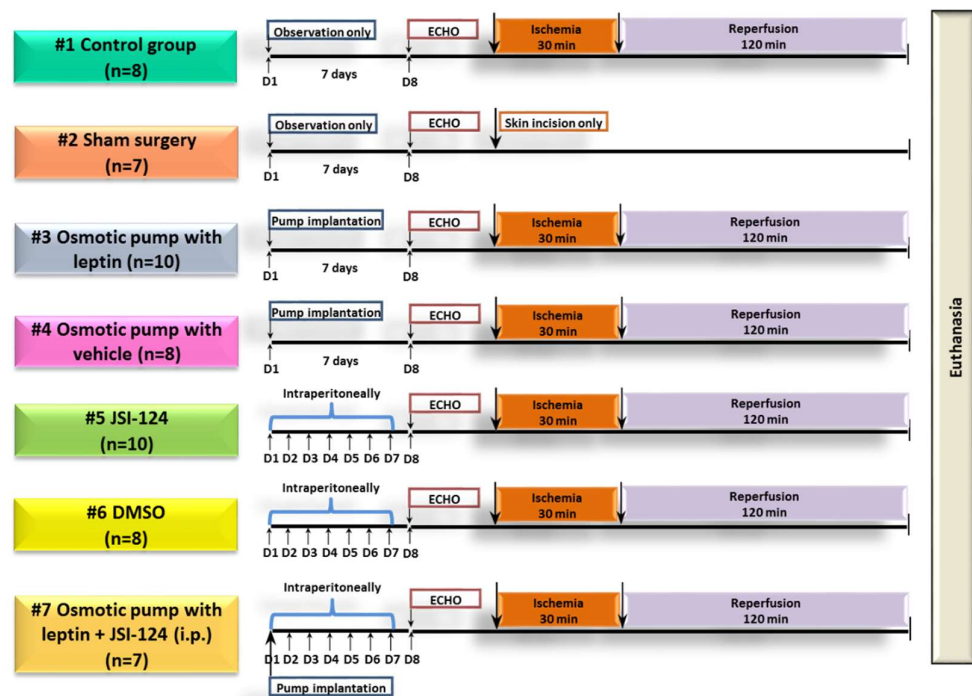


Figure 1. Experimental protocol of the main experimental series aimed at comparison of the cardioprotective effects of hyperleptinemia and JAK/STAT inhibition in the context of post-ischemic and

reperfusion myocardial injury. D, day; ECHO, echocardiography; i.p., intraperitoneal injection; n, number of rats.

Three successive experiments were designed: Experiment 1 (section 2.9.1), 2 (section 2.9.2), and 3 (2.9.3).

4.9.1. Experiment 1

The effects of chronic leptin administration on hemodynamic and metabolic parameters, inflammatory markers, and myocardial IRI Rats were evaluated in four groups: rats received an infusion of either leptin (LEP group) or vehicle (PUMP+VEH group), and the results were compared to the control (CON group) and sham surgery (SH group) groups.

4.9.2. Experiment 2

The impact of long-term treatment with JSI-124 (JAK/STAT inhibitor) on hemodynamic and metabolic parameters, inflammatory markers, and myocardial IRI was evaluated in four groups: rats were randomly allocated to groups which received an intraperitoneal infusion of JSI-124 (JSI-124 group) or vehicle (DMSO group), and the results were compared to the control (CON group) and sham surgery (SH group) groups.

4.9.3. Experiment 3

The effects of JSI-124 treatment on leptin-induced changes in hemodynamic and metabolic parameters, inflammatory markers, and myocardial IRI were evaluated in three groups. Rats were randomly allocated to three different groups: (1) Leptin group: leptin administration without JSI-124 administration (LEP group); (2) JSI-124 group: JSI-124 injected intraperitoneally without leptin administration (JSI-124 group); (3) Leptin + JSI-124 group: JSI-124 was injected intraperitoneally combined with chronic leptin administration (LEP+JSI-124 group).

4.10. Evaluation of biochemical parameters

The plasma levels of leptin (Leptin Rat ELISA Kit; Thermo Fisher Scientific, Inc., Waltham, MA, USA), troponin-I (Rat Cardiac Troponin I ELISA Kit (ab246529); Cambridge, MA, USA), FGF-21 (RayBio® Mouse FGF-21 ELISA Kit; Peachtree Corners, GA, USA), IL-6 (RayBio® Rat IL-6 ELISA Kit; Peachtree Corners, GA, USA), and TNF-alpha (RayBio® Rat TNF-alpha ELISA Kit; Peachtree Corners, GA, USA) were measured using commercial ELISA kits, using specific ELISA kits, according to the manufacturer's instructions. Plasma glucose, total cholesterol, HDL, LDL, and triglyceride concentrations were determined using commercially available kits (Olvex Diagnosticum; Saint-Petersburg, Russia).

4.11. Morphological analysis

Heart apex samples were fixed in 10% buffered formalin, embedded in paraffin, and cut into 4- μ m thick sections. The tissue sections were stained with haematoxylin-eosin (H&E) and examined under a light microscope (Eclipse E400; Nikon, Tokyo, Japan). The size of the cells in the field of view was studied using light microscopy. A morphometric study using the NIS Elements 4.3 Br software. The average cell size was calculated. The results are expressed as mean \pm standard deviation, as there was a normal distribution. All analyses of histological data were performed by two experienced investigators blinded to the treatment groups.

4.12. Statistical analysis

The materials used in this study were statistically processed using parametric and non-parametric methods. The collection, correction, systematisation of the initial information, and visualisation of the obtained results were carried out in Microsoft Office Excel

(2016). Statistical analysis was performed using IBM SPSS Statistics v.26 (IBM Corporation).

Quantitative indicators were assessed against the normal distribution using the Shapiro-Wilk criterion. Combinations of quantitative indicators whose distribution differed from normal were described through the median (Me) and the bottom and top quartiles (Q₁-Q₃). When comparing several samples of quantitative data with a different distribution from the normal, the Kruskal-Wallis criterion was used as a non-parametric alternative to one-factor dispersion analysis. If the calculated value of the Kruskal-Wallis criterion exceeded the critical value, the differences in the indicators were considered to be statistically significant. Otherwise, the null hypothesis was accepted. Depended groups were analysed using Friedman's ANOVA with the Bonferroni post-hoc test. The Kruskal-Wallis test was used to show significant differences between the independent groups. This was followed by the post-hoc Bonferroni test, where significant differences between groups were also identified. All tests were two-tailed, and the significance was set at $p < 0.05$.

A predictive model describing the dependence of the quantitative variable on factors, also represented by quantitative indicators, was developed using the pair or multiple linear regression method to construct the following equation:

$$y = a_0 + a_1x_1 + a_2x_2 + \dots + a_nx_n$$

where y is the result quantitative characteristic, $x_1 \dots x_n$ are the values of the factors measured in the nominal, ordinal, or numerical scale, $a_1 \dots a_n$ are the regression coefficients, and a_0 is a constant.

The resulting regression models make it possible to find the theoretical values of the result characteristic y from the given values of factor x .

The linear correlation coefficient, r_{xy} , was used as an indicator of connectivity. To evaluate the quality of the selection of a linear function, the square of the linear correlation coefficient R^2 , known as the determinism coefficient, was calculated. The determinant coefficient corresponds to the percentage of the factors considered in the model.

5. Conclusions

In conclusion, our results confirm the role of leptin-STAT signalling in mediating leptin-associated mechanisms of metabolic damage and arrhythmogenesis in rats experiencing IRI. Eight-day hyperleptinemia in rats leads to an increase in blood pressure and heart rate, myocardial hypertrophy, impaired LV function, an increase in the frequency of ischemic arrhythmias, dyslipidaemia, systemic inflammation, and an increase in the size of induced myocardial infarction. The plasma leptin level enhanced the size of the infarction by 68.9% in the experimental rat model, and an increase in the level of leptin in blood plasma for every 1 ng/ml was found to result in an increase of the infarct size by 0.193%.

The direct blockade of the JAK/STAT signalling pathway was effective in reversing the negative effects of leptin, including increased blood pressure and total cholesterol, but did not attenuate ischemic myocardial impairment. Based on these findings, we speculate that leptin-STAT signalling may be a good candidate for investigating the aetiology of obesity-related and inflammation-related cardiovascular dysfunctions and heart failure. In addition, these findings also imply that drugs targeting the leptin pathways could provide new treatments for cardiac disorders, especially in obese subjects, in the future.

We showed that hyperleptinemia, coinciding with myocardial ischemia and reperfusion, potentiates myocardial remodelling. This occurs via cardiomyocyte hypertrophy and heart remodelling, and may lead to augmented post-myocardial infarction heart failure. Our rat model simulates a clinical scenario of acute MI with delayed reperfusion, or

myocardial ischemia and reperfusion in patients who suffer from obesity and/or inflammation, which is associated with endogenous leptin induction, for example, in obese patients. Only one dose of JSI-124 was administered.

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