

Evaluating the cardiometabolic impact of Janus-kinase inhibition: An experimental analysis of Tofacitinib's therapeutic potential in a rodent model

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ABSTRACT

Background: Rheumatoid arthritis (RA) is the most prevalent non-infectious inflammatory disorder, often associated with comorbidities such as cardiovascular alterations and metabolic syndrome. These comorbidities significantly influence RA activity, flare duration, and treatment outcomes. This study investigated the effects of the Janus-kinase (JAK) inhibitor on cardiometabolic alterations in the complete Freund's adjuvant (CFA)-induced RA model combined with metabolic syndrome using ZSF1 rats.

Methods: Fifty male ZSF1 rats were divided into lean and obese groups with or without CFA-induced arthritis and tofacitinib treatment.

Inflammation was assessed by paw volume, thermal and mechanical nociceptive testing, and Positron emission tomography (PET) imaging of the tibiotarsal joints using fluorodeoxyglucose ($[^{18}\text{F}]$ FDG). Cardiac effects were assessed with $[^{18}\text{F}]$ FDG-PET scans of the heart and echocardiography. Key metabolic parameters, including lipid metabolism, glucose homeostasis, blood pressure, and body weight, were recorded, followed by histological and molecular analyses.

Results: CFA-induced arthritis led to increased inflammatory and functional cardiac alterations, while metabolic syndrome further aggravated cardiovascular dysfunction. In obese animals, the tofacitinib treatment partially modulated inflammatory and metabolic alterations, whereas in lean animals, it was associated with subtle structural cardiac changes. JAK inhibition did not consistently reduce myocardial fibrosis across experimental groups. Histological analysis confirmed differential patterns of cardiac remodelling between lean and metabolically diseased animals, with more pronounced structural alterations in the presence of metabolic syndrome.

Conclusions: Overall, these findings indicate that JAK inhibition exerts context-dependent effects on cardiac remodelling, suggesting that metabolic status is a key determinant of treatment response in inflammatory arthritis-associated cardiometabolic disease.

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1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease marked by chronic inflammation and a 7.4% rise in global prevalence from 1990 to 2017 [1], representing a growing health burden.

RA doubles cardiovascular risk, while metabolic syndrome (MS) further contributes as a comorbidity [2,3]. Elevated cytokines (tumour necrosis factor alpha: TNF- α , interleukins: IL-1, IL-2, IL-6, IL-23) from synovial inflammation affect distant organs, causing cardiovascular changes, lipid dysregulation, insulin resistance, and muscle adipose replacement, contributing to atherogenesis and mortality [4]. These changes are also common in MS, suggesting an interconnection in the cytokine homeostasis of the two disorders.

MS prevalence in RA patients is 30–40%, depending on disease severity and MS criteria [5,6].

RA treatments, Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), glucocorticoids, and Disease Modifying Antirheumatic Drugs (DMARDs) reduce inflammation but may impair immunity, alter lipid profiles (TNF- α inhibitors, Janus kinase inhibitors), or induce pulmonary fibrosis by blocking IL-6 receptors (tocilizumab) [7,8]. The effects of DMARDs and JAK inhibitors on MS and cardiovascular events remain debated, risk factors must be considered per the latest European Alliance of

Associations for Rheumatology (EULAR) [9]. Particularly, tofacitinib has been less extensively studied regarding its effects on MS and the cardiovascular system [10,11].

In conclusion, the mechanisms by which cytokine levels change and how these alterations in homeostasis influence the progression, morbidity, and mortality of RA patients with MS remain unclear. Understanding the additive effects of these two chronic inflammatory disorders on the immune system could significantly enhance pharmacological therapy, enabling targeted treatment of harmful cytokines. Therefore, this study aims to elucidate the clinically significant cardiometabolic changes associated with RA and MS in addition to commonly used Janus-kinase inhibitor (tofacitinib) treatment.

2. Methods

2.1. Animals

Fifty male Virus Antibody Free (VAF) ZSF1 rats (10 weeks old) were obtained from Charles River Laboratories, Research Models and Services Germany GmbH, Sulzfeld, Germany (Health Certificate No. XXXXX). At baseline (10 weeks of age), lean rats weighed 373.0 ± 15.93 g and obese rats 489.0 ± 22.49 g, while at the end of the experiment, the

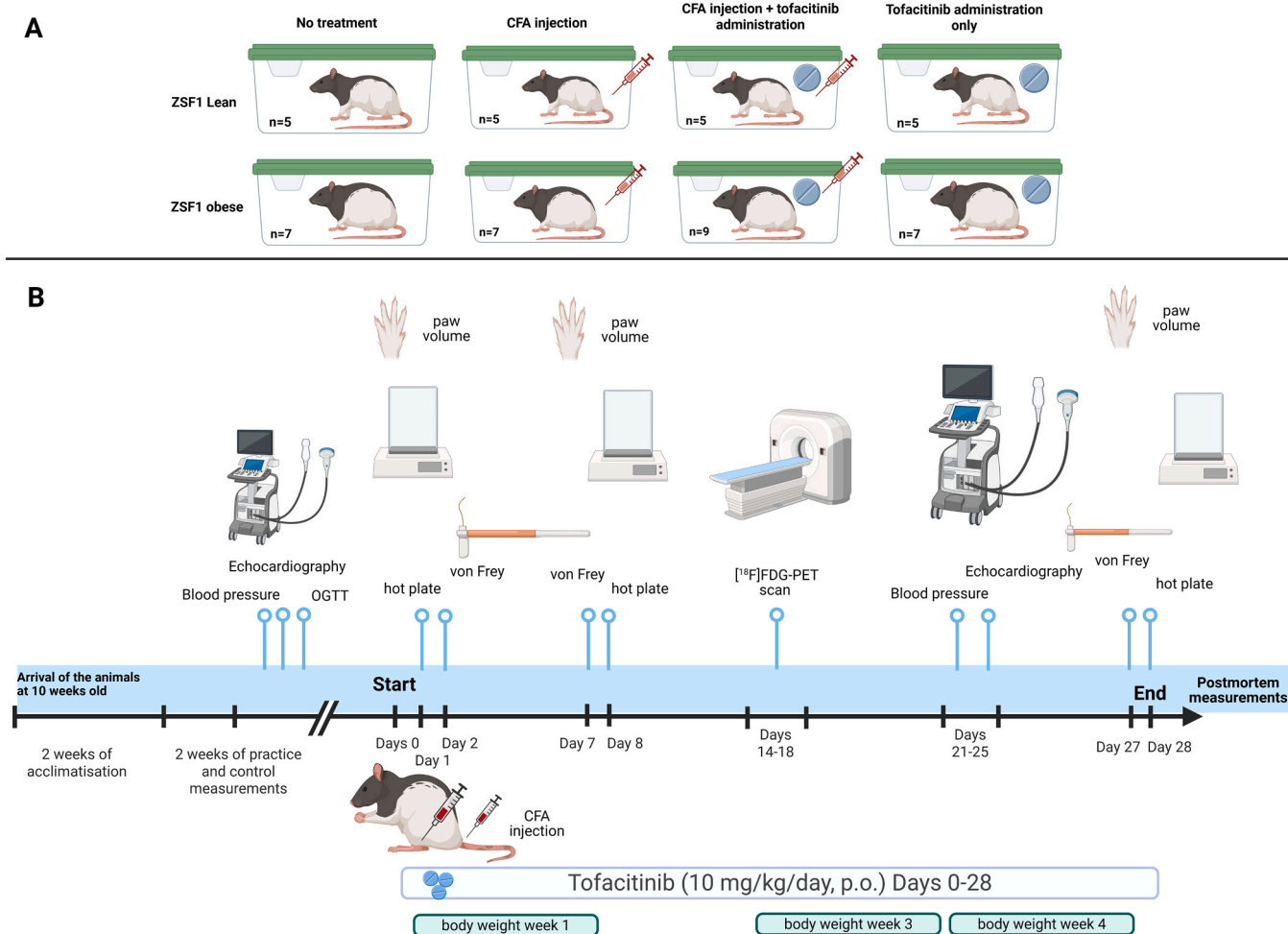


Fig. 1. Experimental design and methodological overview for evaluating the cardiometabolic effects of tofacitinib in ZSF1 rats. A, Group allocation for the in vivo experimental study. ZSF1 lean and obese rats were divided into four subgroups each (n = 5–9/group) based on treatment conditions: no treatment (Control), CFA injection, CFA injection + tofacitinib (Tofa), and tofacitinib administration only. B, Schematic representation of the experimental timeline. After a 2-weeks of acclimatization period, baseline assessments were conducted, including OGTT, body weight, blood pressure, and echocardiography. CFA injection and/or tofacitinib treatment (10 mg/kg/day per os for 28 days) were initiated from Day 0. Functional assessments (paw volume, von Frey, hot plate), [^{18}F]FDG-PET imaging, and metabolic measurements were performed at designated time points. Animals were terminated at the end of the study (Day 28) for post-mortem analyses, including blood biomarker profiling, endothelial function tests, and proteomic analysis of cardiac tissue. Arrows and icons indicate timing and type of each measurement.

corresponding body weights were 392.6 ± 24.1 g and 561.0 ± 25.66 g, respectively (mean $\pm$ SD). The difference between lean and obese groups was statistically significant at both time points ($p < 0.0001$). The sample size was determined using the G-Power program (version 3.1.9.4, Universität Kiel, Germany). A detailed description of the source of the animals and housing conditions can be found in the [supplementary material](#) (Additional File 1.).

2.2. Induction of experimental arthritis

The experimental model involved the administration of 150 μ l of Freund's Complete Adjuvant (CFA, consisting of heat-killed *Mycobacterium tuberculosis* suspended in paraffin oil at 1 mg/ml, Sigma, St. Louis, MO). CFA was injected as described previously ([Fig. 1.](#)) [12].

2.3. Administration of tofacitinib and groups of animals

The animals were divided into eight groups based on the induction of experimental arthritis and the administered treatment. Randomisation was performed manually by an investigator using randomly shuffled animal identifiers. Among these groups, there were two control groups (one lean and one obese) that did not receive either CFA injection or tofacitinib treatment. The Janus kinase inhibitor was administered orally using the gavage technique, after pulverization and dispersion with Mucilago hydroxyethylcellulose (purchased from a local pharmacy). The dose was set at 10 mg/kg body weight per day, as recommended by the literature [13] (Additional File 1., [Fig. 1.](#)). The study concluded on the 28th day with the animals being euthanized through over-anesthesia using ketamine/xylazine (Calypsol Richter Gedeon Nyrt., Budapest, Hungary; Sedaxylan, Eurovet Animal Health B.B., Bladel, Hollandia, ip.: 300/30 mg/kg body weight). Group allocation was performed by an investigator aware of the experimental groups. Experimental procedures were conducted by trained professionals following standardized protocols. Due to technical constraints inherent to specific experimental procedures, identical sample sizes could not be maintained across all experiments. Where the full cohort was not included, the exact number of animals used is explicitly reported in the corresponding figure legends.

2.4. In vivo measurements

2.4.1. Measurement of body weight

Body weight measurements were performed as detailed in the [supplementary material](#) (Additional File 1., [Fig. 1.](#)).

2.4.2. Measurement of paw volume

Measurement of paw volume was performed according to a previously described method, with specific modifications for this study, as detailed in the [supplementary material](#) (Additional File 1., [Fig. 1.](#)) [14].

2.4.3. Measurement of nociceptive thresholds

To evaluate changes in nociceptive thresholds, von Frey and hot plate methods were used according to a previously described method, with specific modifications for this study, as detailed in the [supplementary material](#) (Additional File 1., [Fig. 1.](#)) [15,16].

2.4.4. [^{18} F]FDG-PET scan of the tibiotarsal joints

The examination of the tibiotarsal joints was conducted similarly to the cardiac tissue, as described below. PET images were acquired over the tibiotarsal joint, and Volumes of Interest (VOIs) were placed around this region.

2.4.5. Blood pressure and glucose measurements

Cardiometabolic changes due to CFA and tofacitinib treatments were monitored as detailed in the [supplementary material](#) (Additional File 1.) ([Fig. 1.](#)).

2.4.6. [^{18} F] FDG-PET scan of the hearts

To assess fluorodeoxyglucose uptake in myocardial tissue, [^{18} F] FDG-PET imaging was performed under isoflurane anaesthesia as detailed in the [supplementary material](#) (Additional File 1.) ([Fig. 1.](#)).

2.4.7. Echocardiography

The examination was conducted in the dorsal position under light anaesthesia as detailed in the [supplementary material](#) (Additional File 1.) ([Fig. 1.](#)).

2.5. Ex vivo analysis of the samples

2.5.1. Blood level measurements

Blood samples were collected directly from the heart via cardiac puncture after thoracotomy with the protocol described in the [supplementary material](#) (Additional File 1., [Fig. 1.](#)). HOMA-B (Homeostasis Model Assessment of β -cell function) and HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) were calculated using fasting glucose (mmol/L) and fasting insulin (ng/ml) according to the formula by Matthews et al. and used as a relative index for comparison between groups. Due to assay-dependent variability in insulin measurements (measured by radioimmunoassay according to the manufacturer's protocol: RK-547A150701, Institute of Isotope, Budapest, Hungary), values were not interpreted as absolute physiological thresholds. [17,18].

2.5.2. Endothelial function

Endothelial function tests were performed according to a previously described method, with specific modifications for this study, as detailed in the [supplementary material](#) (Additional File 1., [Fig. 1.](#)) [19].

2.5.3. Cytokine level measurements

After the evaluation of protein concentrations with the method described previously, the proteome profiler cytokine array and western blot analysis were performed with specific modifications for this study, as detailed in the [supplementary material](#) [12] (Additional File 1., [Fig. 1.](#)).

2.5.4. Histological analysis

Histological analysis of the heart samples was performed according to a previously described method, with specific modifications for this study, as detailed in the [supplementary material](#) [19] (Additional File 1., [Fig. 1.](#)).

2.6. Statistical analysis

Statistical analyses were performed using GraphPad Prism software for Windows, version 9.5.1 (La Jolla, CA, USA). Data are presented as the mean $\pm$ standard deviation (SD). The D'Agostino-Pearson normality test was used to assess a Gaussian distribution. For non-Gaussian distributions, the Kruskal-Wallis test followed by Dunn's multiple comparisons test was applied. For Gaussian distributions, 1-, 2-, or 3-way analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparisons test. In all multi-group comparisons, appropriate post hoc tests were used to correct for multiple testing. Probability values (p) less than 0.05 were considered significantly different, indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Outcome assessment and data analysis were performed by a blinded investigator who was unaware of group allocation.

2.7. Funding

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3. Results

The results of the in vivo measurements related to experimental arthritis, including the bodyweight measurements, are presented in Additional File 1. (Supplementary Figures 1., 2., 3.; Supplementary Tables 1., 2.).

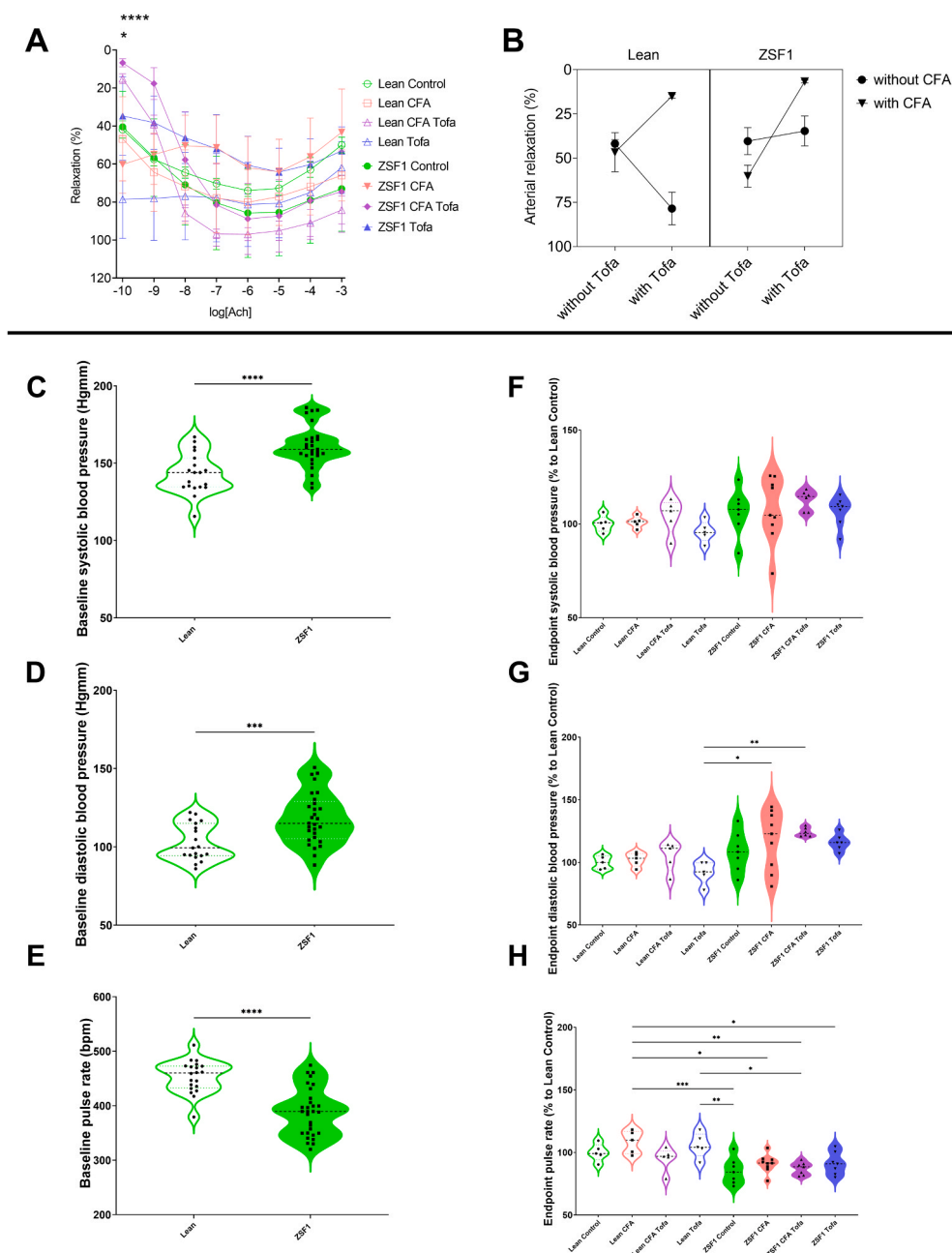


Fig. 2. Endothelial function and cardiometabolic alterations in response to metabolic state, inflammation, and Tofacitinib treatment. A, The endothelium-dependent arterial relaxation in the different groups (mean $\pm$ SD) plotted against the evoking acetylcholine levels (expressed as the common logarithm of the molar concentrations) * $p < 0.05$ **** $p < 0.0001$; $n = 4$ in all lean groups except of Lean Tofa ($n = 5$), $n = 6$ in all obese groups at all acetylcholine concentrations B, changes in the arterial relaxation at the physiological acetylcholine concentration at 0.1 nmol/L Ach concentration (evaluated by three-way ANOVA). C–E, Baseline systolic (C), diastolic (D), and pulse rate (E) measurements in lean versus ZSF1 obese rats, expressed in mmHg or bpm. $n = 20$ in the lean and 30 in the obese groups F–H, Endpoint measurements of systolic (F), diastolic (G), and pulse rate (H) following treatment interventions. Results are expressed as percentage changes relative to the Lean Control group (mean $\pm$ SD.). $n = 5$ in all lean groups, $n = 7$ in ZSF1 Control, ZSF1 CFA Tofa and ZSF1 Tofa groups, $n = 9$ in ZSF1 CFA group. Statistical comparisons were performed using three-way ANOVA (B), unpaired t-probe (C, D, E), one-way ANOVA followed by appropriate post hoc testing (A, F, G, H). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3.1. Hemodynamic and vascular function assessments

3.1.1. endothelial function test

At the physiological Ach concentration reported in the rat plasma (at 0.1 nmol/L) [20] tofacitinib worsened endothelial function in both the lean and obese animals when arthritis (and peri-arthritis) was induced with CFA, although this difference reached the level of statistical significance only in the obese animals (ZSF1 CFA vs. ZSF1 CFA Tofa: $p < 0.0001$). However, without arthritis induced by CFA, tofacitinib improved endothelial function in the lean animals (Lean Control vs. Lean Tofa: $p = 0.0294$), whereas no considerable change was observed between the obese groups (Fig. 2. B, Additional File 1. [Supplementary Table 6.](#)). At higher Ach concentrations (above 1 $\mu\text{mol/L}$), the differences among the treatment groups became smaller (and statistically non-significant) (Fig. 2. A.).

3.1.2. blood pressure and pulse measurements

The ZSF1 animals had significantly higher systolic and diastolic blood pressure even at the beginning of the study, confirming the metabolic syndrome phenotype (Fig. 2. C, D). The diastolic blood pressure increased throughout the study in the ZSF1 CFA and ZSF1 CFA

Tofa groups compared to the Lean Tofa group (Lean Tofa vs. ZSF1 CFA $p = 0.027$, Lean Tofa vs. ZSF1 CFA Tofa $p = 0.0069$) while the systolic blood pressure remained unchanged (Fig. 2. F, G). The pulse rate of the obese animals was lower at the beginning of the study, and this difference persisted by the endpoint measurements too, especially when examining the Lean CFA group compared to all of the obese animals (Lean CFA vs. ZSF1 Control $p = 0.0006$, vs. ZSF1 CFA $p = 0.014$, vs. ZSF1 Tofa $p = 0.022$, vs. ZSF1 CFA Tofa $p = 0.0021$) (Fig. 2. E,H). A significant difference was observed in the OGTT results between lean and obese animals, confirming the presence of metabolic syndrome (Lean vs. ZSF1, $p < 0.0001$) (Additional File 1. [Supplementary Figure 4.](#)).

3.1.3. Comparison of [^{18}F] FDG uptake of the cardiac tissue between lean and ZSF1 groups

[^{18}F] FDG uptake was significantly higher in all Lean groups compared to the ZSF1 groups. A notable difference was observed between the Lean Control and Lean Tofa groups ($p = 0.0477$). In the obese groups, radiotracer accumulation was significantly increased in response to CFA treatment and the combination of treatments, whereas tofacitinib alone did not affect the glucose homeostasis of the hearts in

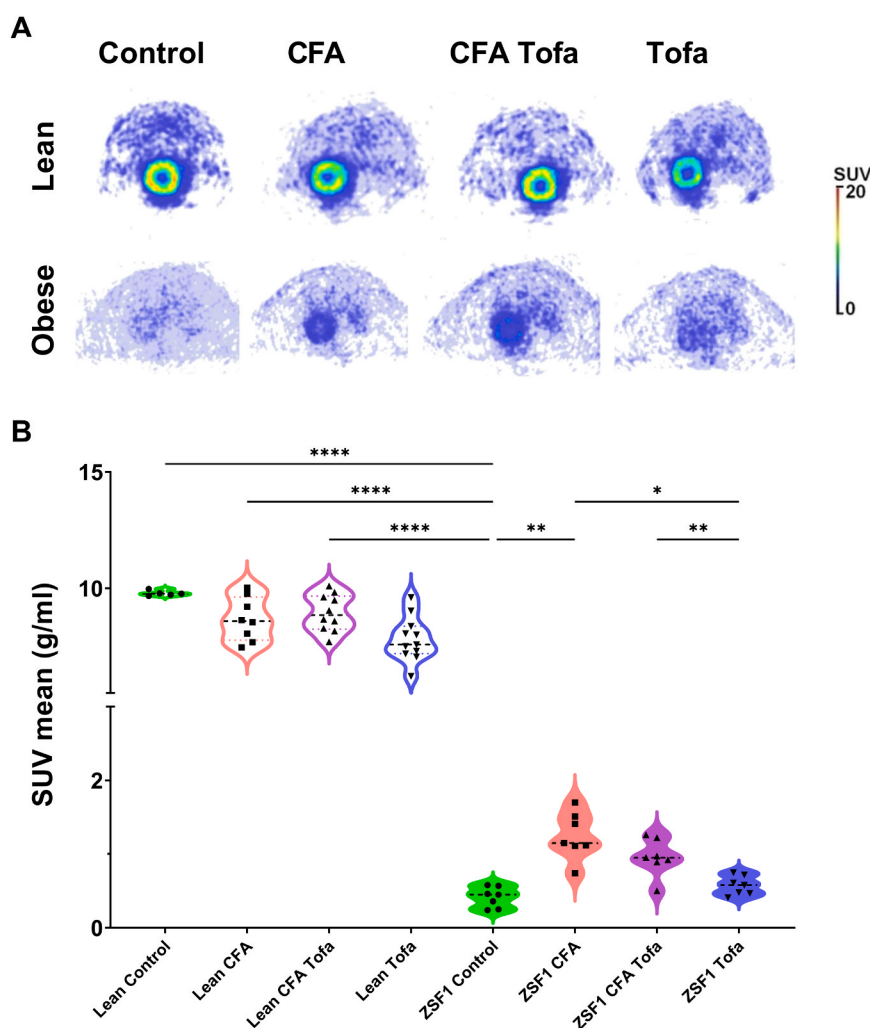


Fig. 3. Alterations in myocardial glucose uptake assessed by [^{18}F]FDG-PET Imaging. A, Representative transverse PET images of myocardial [^{18}F]FDG uptake across experimental groups, illustrating differences between lean and obese (ZSF1) rats in the control, CFA-induced inflammation, tofacitinib (Tofa) treatment, or combined CFA Tofa conditions. The scale represents standardized uptake values (SUV; range: 0–20). B, Quantitative analysis of myocardial SUV mean (g/ml) among the treatment groups. Data are presented as mean $\pm$ SD. Statistical comparisons were conducted using mixed-effects analysis followed by appropriate post hoc testing. Significance levels are indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Number of values were 5 in the Lean Control, 8 in the Lean CFA, 10 in the Lean CFA Tofa, 11 in the Lean Tofa groups, and 7 in all obese groups.

obese animals (ZSF1 Control vs. ZSF1 CFA, $p = 0.0078$; ZSF1 CFA vs. ZSF1 Tofa, $p = 0.0245$; ZSF1 CFA Tofa vs. ZSF1 Tofa, $p = 0.0044$) (Fig. 3.).

3.1.4. Evaluation of systolic and diastolic function

To assess systolic function, ejection fraction (EF) was measured, revealing significantly higher levels in the ZSF1 Control (Lean Control vs. ZSF1 Control, $p = 0.0200$) and ZSF1 CFA groups compared to the Lean Control (Lean Control vs. ZSF1 CFA, $p = 0.0004$). The difference was even more pronounced when comparing the CFA-treated obese animals to the Lean CFA group (Lean CFA vs. ZSF1 CFA, $p < 0.0001$). Differences were also detected between the Lean CFA and Lean CFA Tofa groups ($p = 0.0033$) (Fig. 4. C).

The diameter of the posterior wall of the left ventricle was highest in the ZSF1 CFA group (mean $\pm$ SD: 2.452 ± 0.09462), showing a significant difference compared to the Lean CFA, Lean Control, ZSF1 CFA Tofa, and ZSF1 Tofa groups (ZSF1 CFA vs. Lean CFA, $p = 0.0161$; vs. Lean Control, $p = 0.0002$; vs. ZSF1 CFA Tofa, $p = 0.0001$; vs. ZSF1 Tofa, $p = 0.0002$). In the Lean CFA Tofa group, the posterior wall of the left ventricle was significantly thicker compared to the ZSF1 Tofa and ZSF1 CFA Tofa groups (Lean CFA Tofa vs. ZSF1 Tofa, $p = 0.0046$; vs. ZSF1 CFA Tofa, $p = 0.0041$). Among obese animals, both the Control and CFA-treated groups exhibited greater posterior wall thickness compared to their respective lean counterparts (ZSF1 Control vs. Lean Control, $p = 0.0234$; ZSF1 CFA vs. Lean CFA, $p = 0.0161$). However, in obese animals treated with tofacitinib solely, lower values were observed compared to the ZSF1 Control group (ZSF1 Tofa vs. ZSF1 Control, $p = 0.0328$). In the Lean CFA group, LVpWd was higher compared to the control (Lean CFA Tofa vs. Lean Control, $p = 0.0035$) (Fig. 4. D.).

In line with the previous results, the relative wall thickness (RWT) of obese CFA-induced animals was significantly higher compared to the identical Lean, to the Lean Control, and to both treated obese groups (ZSF1 CFA vs. Lean CFA, $p = 0.0403$ vs. Lean Control, $p = 0.0014$ vs. ZSF1 CFA Tofa, $p = 0.0003$ vs. ZSF1 Tofa $p = 0.0003$). This parameter was the highest in the Lean CFA Tofa group, measurable differences were observed compared to the Lean Control, ZSF1 Tofa, and ZSF1 CFA Tofa groups (Lean CFA Tofa vs. Lean Control, $p = 0.0044$, vs. ZSF1 Tofa, $p = 0.0016$, vs. ZSF1 CFA Tofa, $p = 0.0015$) (Fig. 4. E).

Regarding diastolic function, MV DecT was shorter in the Lean Control group compared to the Lean Tofa and ZSF1 Control groups (Lean Control vs. Lean Tofa $p = 0.0003$ vs. ZSF1 Control, $p < 0.0001$), and a significant decrease was observed in the CFA-treated lean group due to tofacitinib treatment (Lean CFA vs. Lean CFA Tofa, $p = 0.0326$). The mitral valve deceleration time appeared to be the highest in the ZSF1 Tofa group (ZSF1 Control: 54.86 ± 3.364 ms vs Lean Control: 30.40 ± 3.234 ms, $p < 0.0001$) (Fig. 4. H.).

IVRT was shortest in the Lean Control group compared to other Lean animal groups (Lean Control vs. Lean CFA, $p = 0.0037$ vs. Lean CFA Tofa, $p = 0.0209$ vs. Lean Tofa, $p = 0.0112$). The highest rates were observed in the Lean CFA (mean: 35.80 ± 2.131) and ZSF1 CFA groups (mean: 35.11 ± 1.274), with a significant difference between the Lean Tofa and ZSF1 Tofa groups (Lean Tofa vs. ZSF1 Tofa, $p = 0.0003$) (Fig. 4. I.).

The Tei-index was higher in the Lean CFA and ZSF1 CFA groups compared to their respective control groups (Lean Control vs. Lean CFA, $p = 0.0155$; ZSF1 Control vs. ZSF1 CFA, $p = 0.0024$) and significantly lower in the ZSF1 CFA Tofa group compared to the ZSF1 CFA group (ZSF1 CFA vs. ZSF1 CFA Tofa, $p = 0.0029$). This parameter was the lowest in the obese tofacitinib-treated group (mean: 0.3910 ± 0.02068) (Fig. 4. J.).

No differences were detected in the E/A ratio (Fig. 4. E). The E/e' ratio was significantly higher in the ZSF1 CFA Tofa group than in the Lean Control group ($p = 0.0045$), whereas the e'/a' ratio was found to be the lowest following the combination treatments in both lean and obese animals (Lean CFA Tofa vs. Lean Tofa, $p = 0.0007$; vs. Lean Control, $p = 0.0441$; ZSF1 CFA Tofa vs. ZSF1 Tofa, $p = 0.0001$) (Fig. 4.

K, L, M).

3.1.5. Results of blood tests regarding cardiac function

The CK levels of the animals remained within normal limits without any observable differences (Additional File 1. [Supplementary Figure 4.](#)). However, CK-MB levels were reduced in obese animals following tofacitinib treatment (ZSF1 Control vs. ZSF1 Tofa $p = 0.0420$), as was observed with LDH (ZSF1 Control vs. ZSF1 Tofa $p = 0.0312$) (Fig. 5. A, B).

3.1.6. Results of blood tests regarding metabolic syndrome

Total cholesterol levels were significantly elevated in all obese animals compared to the Lean Control group ($p < 0.0001$). Neither CFA nor tofacitinib, either alone or in combination, altered these levels. No differences were observed in the colourimetric pancreatic lipase assay. However, triglyceride levels were higher in ZSF1 animals across all treatment groups compared to their Lean counterparts, except for the control groups (Lean Tofa vs. ZSF1 Tofa $p = 0.0105$, Lean CFA vs. ZSF1 CFA $p = 0.0174$, Lean CFA Tofa vs. ZSF1 CFA Tofa $p = 0.0163$). HDL levels in ZSF1 animals were consistently higher in all groups compared to Lean animals (Lean Control vs. ZSF1 Control $p < 0.0001$, Lean CFA vs. ZSF1 CFA $p < 0.0001$, Lean CFA Tofa vs. ZSF1 CFA Tofa $p < 0.0001$, Lean Tofa vs. ZSF1 Tofa $p < 0.0001$), in the obese animals, tofacitinib treatment resulted in a higher HDL level (ZSF1 Control vs. ZSF1 Tofa $p = 0.0394$). A significant difference in LDL levels was observed between Lean Tofa and ZSF1 Tofa animals ($p = 0.0137$) (Table 1.).

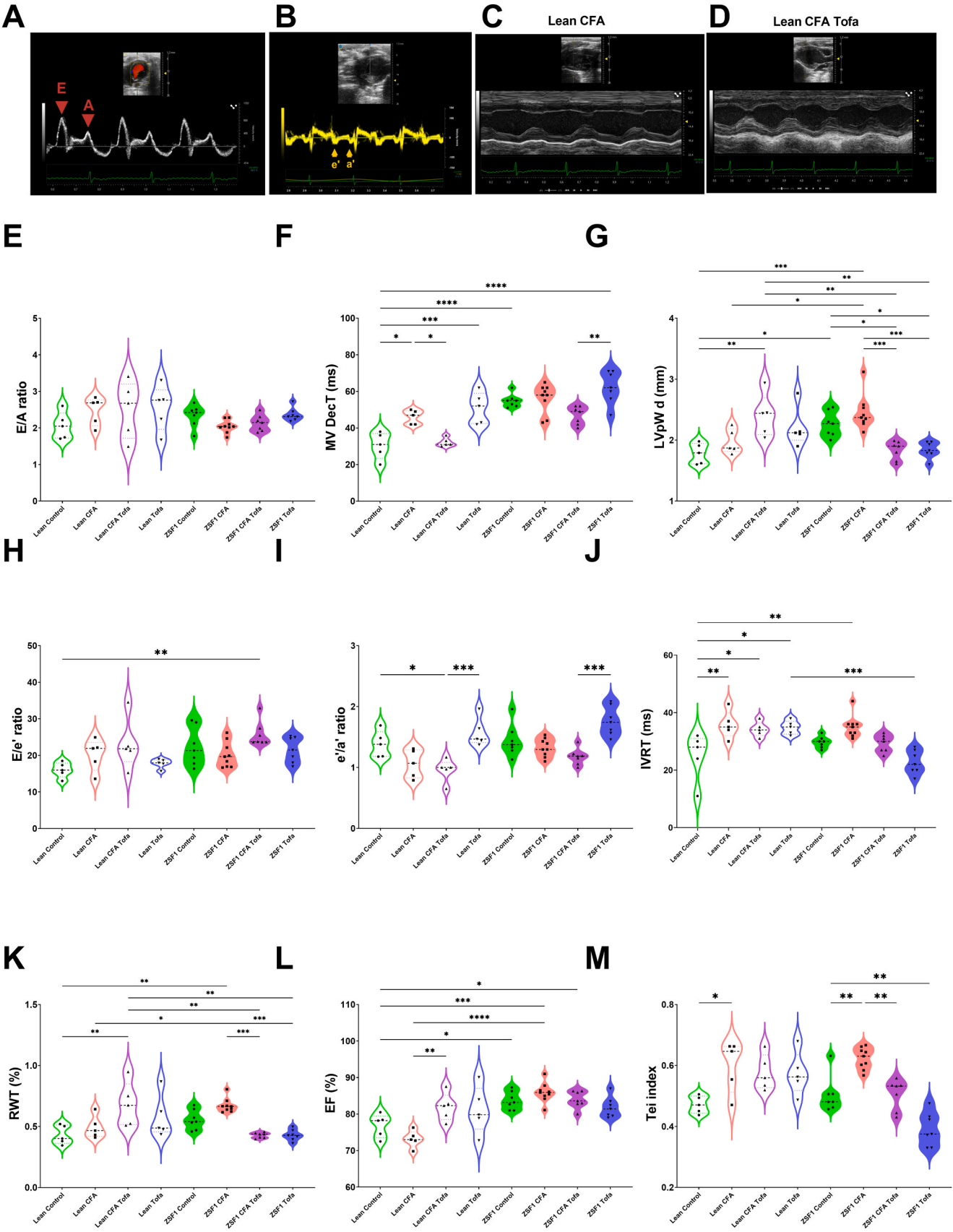
Regarding other parameters connected to the liver function, characteristic differences were observed due to MS (Additional File1. [Supplementary Table 4.](#)).

All ZSF1 animals exhibited elevated glucose levels compared to their respective Lean groups (Lean Control vs. ZSF1 Control $p = 0.0192$, Lean CFA vs. ZSF1 CFA $p = 0.0488$, Lean CFA Tofa vs. ZSF1 CFA Tofa $p < 0.0001$, Lean Tofa vs. ZSF1 Tofa $p < 0.0001$). Neither CFA nor tofacitinib, alone or combined, affected blood glucose levels (Fig. 5. C). Additionally, there were no changes observed in fructosamine and HbA1C levels across the study period (Additional File 1. [Supplementary Table 5.](#)).

The HOMA-IR and HOMA- β indices were calculated to assess insulin resistance and pancreatic β -cell function, respectively. ZSF1 Control animals exhibited significantly higher HOMA-IR values compared with their lean counterparts ($p = 0.0376$). This disparity between the lean and obese phenotypes remained evident following CFA, tofacitinib, or combination treatment (ZSF1 Control vs. Lean CFA $p = 0.0376$, vs. Lean CFA Tofa $p = 0.0007$, vs. Lean Tofa $p = 0.0003$). Furthermore, significant differences were observed between lean and obese animals receiving either tofacitinib monotherapy or combination treatment (Lean CFA Tofa vs. ZSF1 CFA Tofa $p = 0.0004$, Lean Tofa vs. ZSF1 Tofa $p = 0.0010$). Interestingly, the Lean Tofa group exhibited significantly higher HOMA-B values compared with all other lean groups, including Lean Control ($p < 0.0001$), Lean CFA ($p < 0.0001$), and Lean CFA Tofa ($p = 0.0151$). Moreover, HOMA-B values in the Lean Tofa group were significantly greater than those observed in both ZSF1 Control ($p < 0.0001$) and ZSF1 Tofa ($p < 0.0001$) groups. In addition, the Lean CFA Tofa group demonstrated higher HOMA-B values than the ZSF1 Control group ($p = 0.0385$) (Fig. 6.).

3.1.7. Alterations in cardiomyocyte diameter and fibrotic area of cardiac tissue sections

Compared to the Lean Control group, cardiomyocyte size was significantly increased in ZSF1 Control animals ($p < 0.0001$). In lean animals, both CFA and tofacitinib treatments increased cardiomyocyte diameter compared to the control group (Lean Control vs. Lean CFA, Lean Tofa, $p < 0.0001$), and the Janus kinase inhibitor reduced the enlargement induced by CFA (Lean CFA vs. Lean CFA Tofa, $p < 0.0001$). Among lean groups, the largest cardiomyocyte diameter was observed in the tofacitinib-treated groups (mean $\pm$ SD: 14.55 ± 0.1895). In obese



(caption on next page)

Fig. 4. Effects of CFA-induced inflammation and tofacitinib treatment on left ventricular diastolic and systolic function as assessed by echocardiography. A, Representative pulsed-wave (PW) Doppler image showing early (E) and late (A) transmitral filling velocities in an obese rat treated with tofacitinib solely B, Representative tissue Doppler image at the septal mitral annulus, displaying early (e') and late (a') diastolic myocardial velocities of a CFA and tofacitinib treated lean rat. C,D Representative M-mode echocardiographic traces (PSLAX view) of the left ventricle in lean rats with CFA A, and CFA + tofacitinib (Tofa) treatment B; E, F, G, Quantitative analysis of echocardiographic parameters related to systolic function: E, Ejection fraction (EF, %); F, Mitral valve deceleration time (MV DecT, ms); G, Left ventricular posterior wall thickness in diastole (LVPWd, mm); H, I, J, Quantitative analysis of echocardiographic parameters related to diastolic function: H, E/e' ratio, reflecting LV filling pressure; I, e'/a' ratio, indicative of myocardial relaxation. J, Isovolumic relaxation time (IVRT, ms); K, Relative wall thickness (RWT), calculated as $(2 \times \text{LVPWd}) / \text{LVIDd}$; L, E/A ratio M, Myocardial performance index (Tei index), calculated as $(\text{IVRT} + \text{IVCT}) / \text{LVET}$. Data are expressed as mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. $n = 5$ in all lean groups, $n = 7$ in ZSF1 Control, ZSF1 CFA Tofa and ZSF1 Tofa groups, $n = 9$ in ZSF1 CFA group.

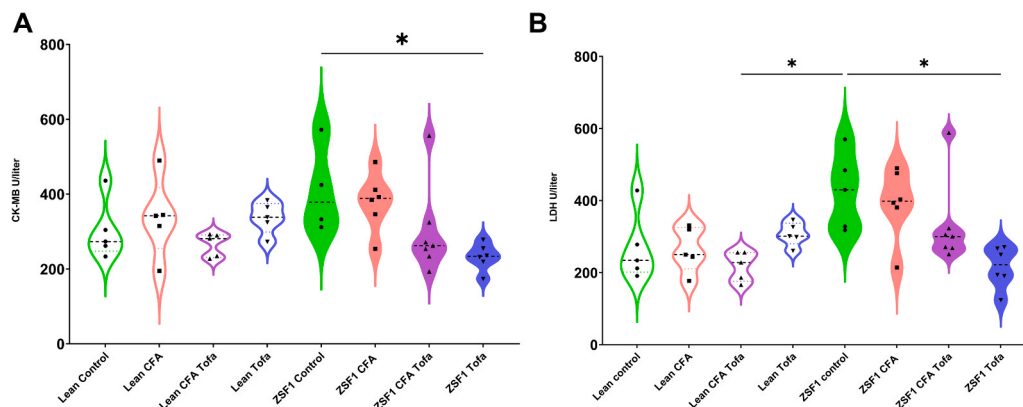


Fig. 5. Blood parameters reflecting heart function and metabolic alterations. A, Serum levels of CK-MB, $n = 5$ in all lean groups, $n = 4$ in ZSF1 CFA and ZSF1 Tofa groups, $n = 7$ in ZSF1 CFA Tofa group B, LDH in response to CFA and/or tofacitinib treatment. $n = 5$ in all lean groups, $n = 5$ in ZSF1 Control, $n = 6$ in ZSF1 CFA and ZSF1 Tofa groups, $n = 7$ in ZSF1 CFA Tofa group Data are presented as mean $\pm$ SD. Statistical significance was assessed by one-way ANOVA; * $p < 0.05$, except for CK levels, where Kruskal-Wallis appeared to be the accurate test. Parameters were analysed using one-way ANOVA. Data are expressed as mean $\pm$ SD. Statistical significance: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

Table 1

Blood parameters reflecting metabolic alterations.

	CHOL (mmol/L)			LIPC (U/L)			Triglyceride (mmol/L)		
	mean	SD		mean	SD		mean	SD	
Lean Control	1.56	0.13		5.00	0.19		0.89	0.15	
Lean CFA	1.47	0.03		4.82	0.63		0.39	0.03	vs. ZSF1 CFA *
Lean CFA Tofa	1.64	0.03		4.78	0.22		0.39	0.18	vs. ZSF1 CFA Tofa *
Lean Tofa	1.57	0.27		4.64	0.18		0.34	0.06	vs. ZSF1 Tofa *
ZSF1 Control	4.43	0.89	vs. Lean Control ****	7.32	2.77		8.79	2.27	
ZSF1 CFA	4.42	0.56	vs. Lean Control ****	5.38	0.23		10.07	1.56	
ZSF1 CFA Tofa	5.31	1.26	vs. Lean Control ****	5.59	0.44		9.56	1.16	
ZSF1 Tofa	5.35	0.84	vs. Lean Control ****	5.78	0.43		10.50	1.43	
	HDL (mmol/L)			LDL (mmol/L)			Glucose (mmol/L)		
	mean	SD		mean	SD		mean	SD	
Lean Control	1.17	0.1	vs. ZSF1 control ****	0.25	0.03		17.60	1.95	vs. ZSF1 control *
Lean CFA	1.09	0.05	vs. ZSF1 CFA ****	0.30	0.02		15.01	0.70	vs. ZSF1 CFA *
Lean CFA Tofa	1.30	0.05	vs. ZSF1 CFA Tofa ****	0.23	0.03		9.47	0.70	vs. ZSF1 CFA Tofa ****
Lean Tofa	1.16	0.2	vs. ZSF1 Tofa ****	0.22	0.07	vs. ZSF1 Tofa *	7.60	1.59	vs. ZSF1 Tofa *
ZSF1 Control	1.99	0.39	vs. ZSF1 Tofa *	0.33	0.18		28.82	9.58	
ZSF1 CFA	1.84	0.17		0.18	0.03		24.29	3.78	
ZSF1 CFA Tofa	2.12	0.33		0.30	0.10		25.76	6.96	
ZSF1 Tofa	2.44	0.23		0.43	0.15		25.54	5.97	

Metabolic blood profile including total cholesterol (CHOL), colorimetric pancreatic lipase assay (LIPC), triglycerides, HDL, LDL, and glucose levels across all experimental groups. Kruskal-Wallis test was used for triglyceride levels; all other parameters were analyzed using one-way ANOVA. Data are expressed as mean $\pm$ SD. Statistical significance: * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

groups, CFA injection increased cardiomyocyte diameter (ZSF1 Control vs. ZSF1 CFA, $p < 0.0001$), and tofacitinib treatment reduced it (ZSF1 CFA vs. ZSF1 CFA Tofa, $p < 0.0001$), although it remained higher than in the ZSF1 Control group (ZSF1 Control vs. ZSF1 CFA Tofa, $p < 0.0001$) (Fig. 7. B.).

There were no significant differences in fibrotic areas among lean animals; however, the tofacitinib-treated group showed a slight increase in fibrosis compared to the Lean Control group. In all obese animals,

except for ZSF1 Control, cardiac tissue exhibited higher fibrosis compared to the Lean control group (Lean Control vs. ZSF1 CFA, $p = 0.0029$; ZSF1 CFA Tofa vs. Lean Control, $p = 0.0004$; ZSF1 Tofa vs. Lean Control, $p = 0.0116$). Neither CFA nor tofacitinib treatments had pronounced effects on fibrosis (Fig. 7. C.).

3.1.8. Results of cytokine array

Of the 79 examined cytokines, seven showed significant differences

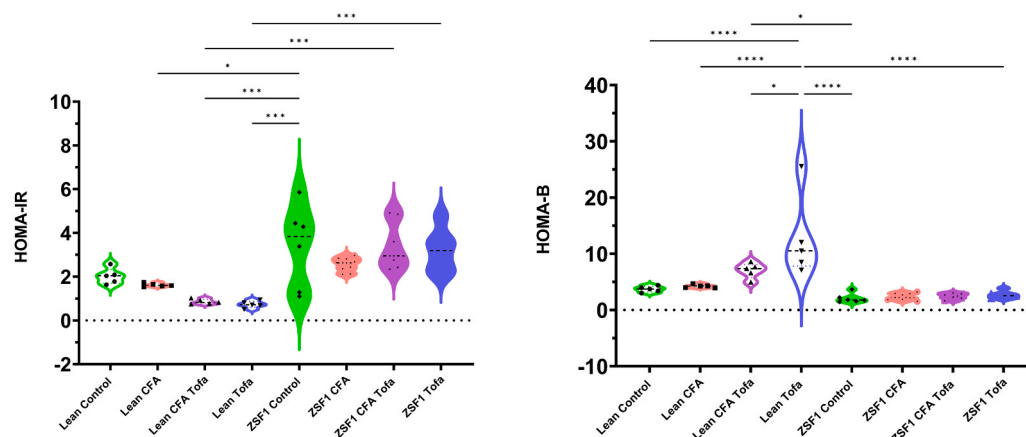


Fig. 6. HOMA-IR and HOMA-B indices across experimental groups. HOMA-IR, an index of systemic insulin resistance, and HOMA-B, an estimate of pancreatic β -cell function, were calculated in lean and obese ZSF1 rats under control conditions and following CFA, tofacitinib, or combined treatments. Data are presented as mean $\pm$ SD. Statistical comparisons between groups are indicated as * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$, $n = 5$ in all lean groups, $n = 6$ in ZSF1 Control and ZSF1 Tofa groups, $n = 7$ in ZSF1 CFA Tofa group, $n = 9$ in ZSF1 CFA group.

in the treatment groups. In the CYR-61 and CNTF levels, similar differences were observed between the tofacitinib-treated lean and obese animals (Lean Tofa vs. ZSF1 Tofa CYR-61 $p = 0.0166$, CNTF $p = 0.0094$). Examining the Resistin levels of the animals, significant differences were observed. The Lean CFA Tofa group had significantly higher expression than the ZSF1 CFA Tofa, the Lean Tofa group had significantly higher expression than the ZSF1 Control and ZSF1 Tofa groups (Lean CFA Tofa vs. ZSF1 CFA Tofa $p = 0.0245$, Lean Tofa vs. ZSF1 Control $p = 0.0043$, Lean Tofa vs. ZSF1 Tofa $p = 0.0038$). Regarding the cytokine IL-22, significantly lower expression was observed in the CFA and tofacitinib-treated animals (ZSF1 Control vs. Lean CFA $p = 0.0395$, vs. Lean Tofa $p = 0.0206$). RAGE had a significantly higher level in the ZSF1 CFA group compared to the Lean CFA ($p = 0.0382$). In the tofacitinib-treated group, the cytokine VEGF-A was expressed significantly higher than in the obese group (Lean Tofa vs. ZSF1 Tofa $p < 0.0001$). The CCL5 level was lower in the tofacitinib-treated CFA-induced obese animals compared to the obese animals suffering from the symptoms of experimental arthritis (ZSF1 CFA vs. ZSF1 CFA Tofa $p = 0.0057$) (Fig. 8. A.).

3.1.9. Results of Western blot analysis

Among the cytokines investigated in relation to the Janus kinase system, a significant difference was noted in the expression of CNTF. The CNTF level was higher in the Lean Tofa group compared to obese animals with arthritis (Lean Tofa vs. ZSF1 CFA, $p = 0.0466$). Although slight increases were observed in NRF2 in the CFA and/or Tofacitinib groups, these differences did not reach statistical significance. The difference in TGF- β 1 expression between the Lean Control and Lean Tofa groups approached statistical significance ($p = 0.0523$). A significant increase in the relative expression of TGF- β 1 was observed in the Lean CFA Tofa group compared to the Lean Control group (Lean CFA Tofa vs. Lean Control, $p = 0.0204$). (Fig. 9.B.). To further investigate the underlying mechanisms, the nuclear expression levels of STAT3 and NF- κ B were assessed by Western blot analysis. However, no significant differences were observed between the experimental groups (Fig. 9. A.).

4. Discussion

Our research team investigated the impact of the FDA-approved Janus kinase inhibitor tofacitinib on the progression of experimentally induced arthritis. We used a complete Freund's adjuvant (CFA) animal model to induce systemic inflammation and chronic polyarthritis mimicking autoimmune arthritis, suitable for studying inflammation-driven cytokine and organ changes [21]. Metabolic syndrome also

involves chronic inflammation and altered cytokine levels, yet their shared pathways remain incompletely understood [22,23].

4.1. Metabolic syndrome in the present study

The ZSF1 rat model closely mimics MS, meeting four of the five criteria: hyperglycemia, hypertension, dyslipidemia, and abdominal obesity [24]. These metabolic alterations were reflected in the marked differences in metabolic and cardiovascular parameters between lean and obese animals throughout the study.

4.1.1. Systemic metabolic profile

Obese animals exhibited elevated HDL levels, as typical in rats, but opposite to human patterns [25]. Triglyceride and total cholesterol levels differed significantly but were unaffected by treatments (Fig. 5. C.). Tofacitinib's influence on LDL and HDL levels, particularly in obese animals, aligns with our findings [26,27].

While previous studies reported no weight effect [28], our study confirms reduced weight gain in animals with normal metabolism (Additional File 1. Supplementary Figure 1, Supplementary Tables 1., 2.). In rheumatoid cachexia, elevated TNF- α increases fat mass, while reducing muscle, yielding an unchanged BMI but higher morbidity [29, 30]. Both RA and MS increase adipose tissue, perpetuating inflammation [31].

Tofacitinib's reported impact on blood pressure is inconsistent [10, 27]. In our study, it caused no significant changes except a mild diastolic drop in lean Tofa animals (Fig. 2. G.).

4.1.2. Glucose homeostasis

Insulin resistance and type-2 diabetes are prominent features of MS in the ZSF1 model. Obese rats showed persistent hyperglycaemia and elevated HOMA-IR, confirming significant insulin resistance. In contrast, increased HOMA-B in Lean Tofa animals may indicate enhanced β -cell function or compensatory insulin secretion under metabolically healthy conditions. This effect was absent in obese animals, implying that obesity-related metabolic disturbances may blunt potential treatment-related improvements in β -cell function. As HOMA-B is an indirect marker of β -cell activity, the finding should be interpreted with caution, and further mechanistic studies are warranted (Fig. 6.).

In contrast to the systemic insulin resistance reflected by HOMA-IR, [^{18} F] FDG-PET scan results potentially indicate improved insulin sensitivity and enhanced glucose utilization [32]. Cardiac [^{18} F] FDG-PET scan methodology revealed glucose metabolism performed under

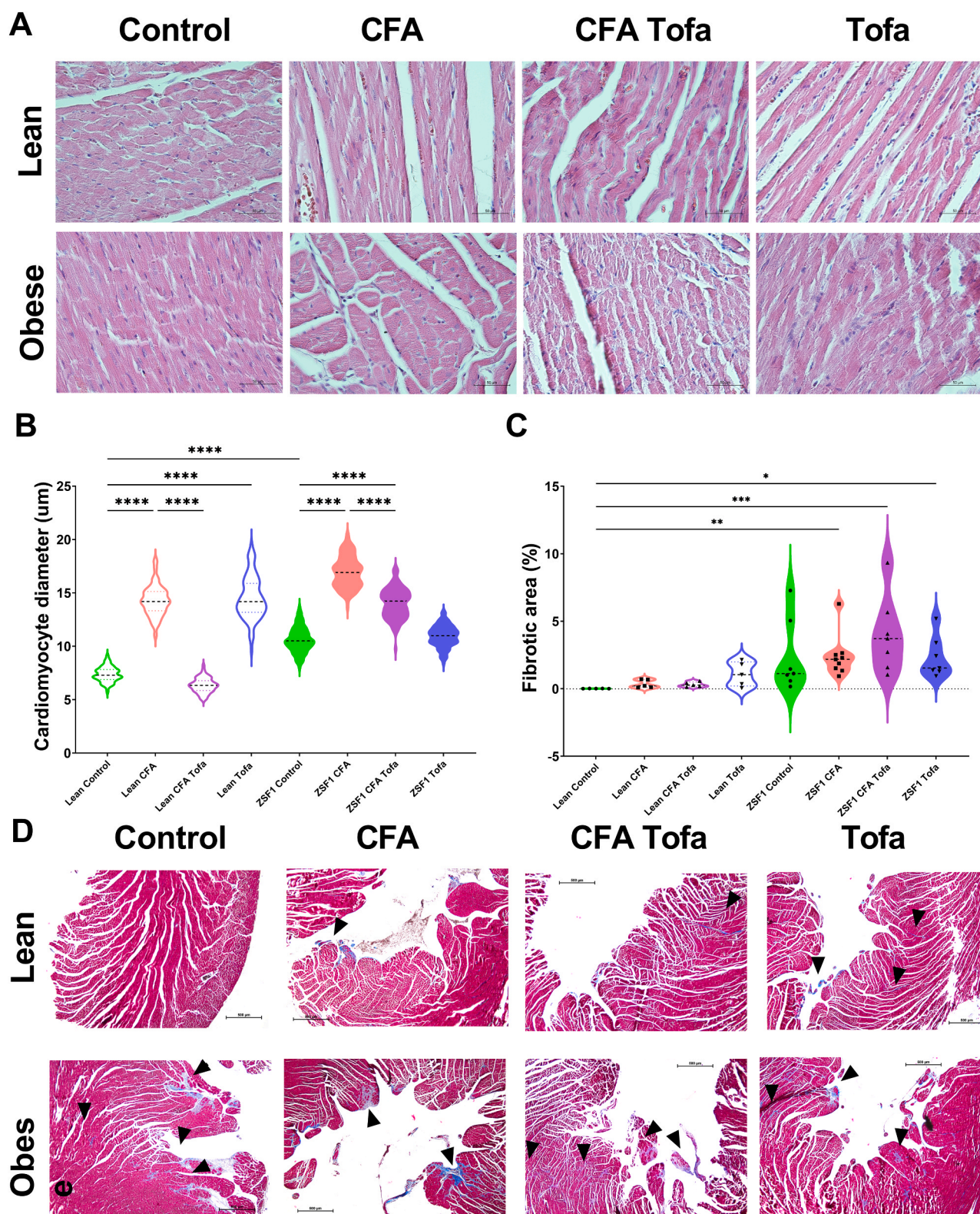


Fig. 7. Histological evaluation of experimental arthritis and tofacitinib treatment on cardiac morphology and fibrosis. A, Representative hematoxylin and eosin (H&E)-stained sections of left ventricular myocardium in lean and obese (ZSF1) rats across treatment groups (Control, CFA, CFA Tofa, Tofa) (magnification: 400 ×; scale bars shown). B, Quantification of cardiomyocyte diameter. Cardiomyocyte size was significantly increased in ZSF1 Control rats and in Lean rats following CFA or Tofa treatment. Statistical analysis was performed using the Kruskal–Wallis test; **** $p < 0.0001$; 100 cells were measured by each animal, $n = 500$ in all lean groups, $n = 700$ in ZSF1 Control, ZSF1 CFA Tofa and ZSF1 Tofa groups, $n = 900$ in ZSF1 CFA group C, Quantification of myocardial fibrosis (% fibrotic area) across treatment groups. Fibrosis was markedly elevated in ZSF1 rats compared to Lean Controls, with the highest levels observed in the ZSF1 CFA Tofa group. Statistical analysis was performed using the Kruskal–Wallis test; ** $p < 0.01$, $n = 5$ in all lean groups, $n = 7$ in ZSF1 Control, ZSF1 CFA Tofa and ZSF1 Tofa groups, $n = 9$ in ZSF1 CFA group D, Representative images of Masson's trichrome-stained cardiac sections showing interstitial and perivascular fibrosis (indicated by black arrowheads) in Lean and ZSF1 rats across treatment groups (magnification: 40 ×; scale bars shown).

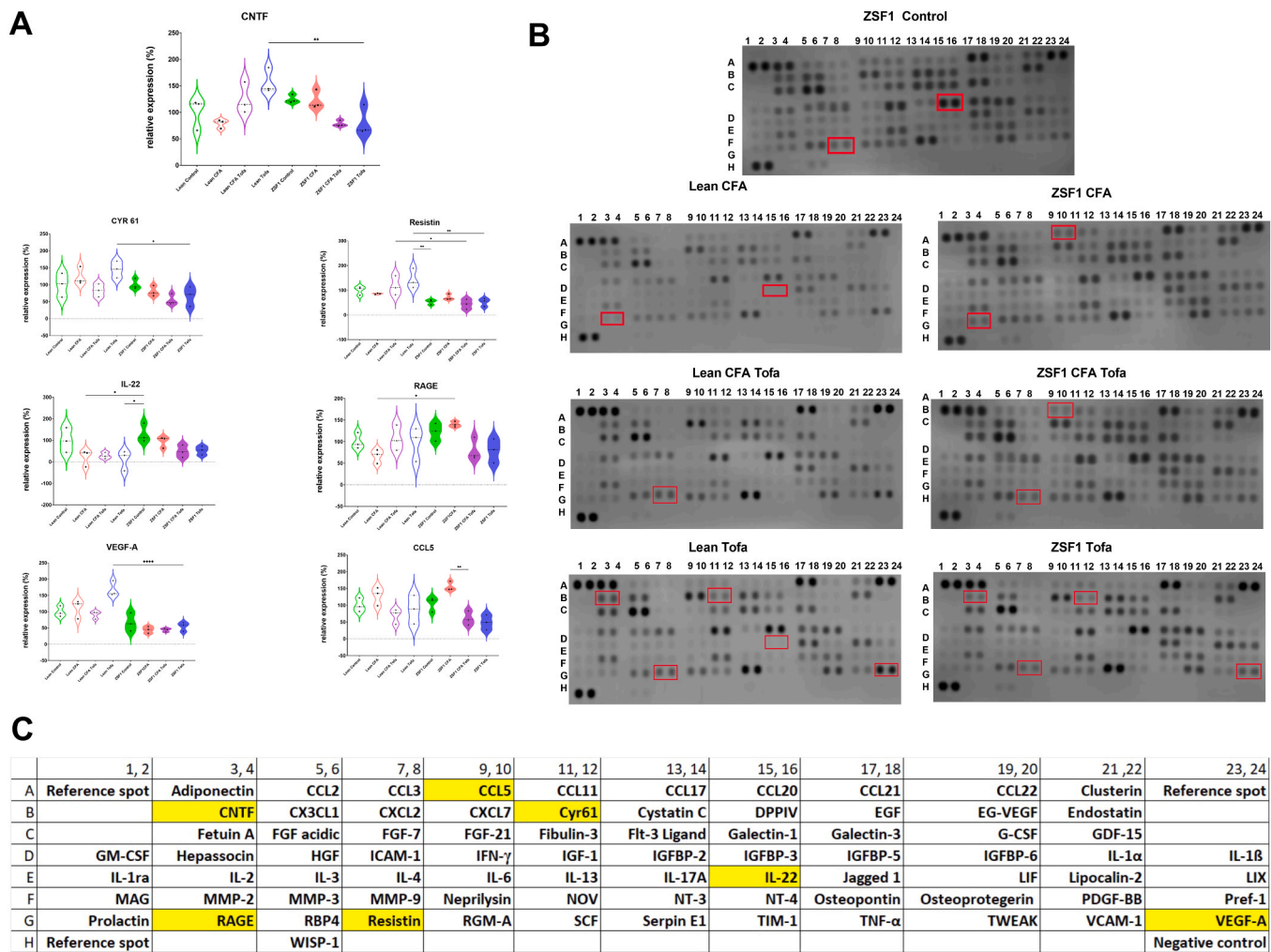


Fig. 8. Cardiac cytokine profile and protein expression in response to experimental arthritis and tofacitinib treatment examined via protein array. A, Quantitative analysis of cytokines identified by array analysis. Relative concentrations (%) of CNTF, CYR61, Resistin, IL-22, RAGE, VEGF-A, and CCL5 were quantified from cardiac tissue lysates. Significant alterations were observed among groups. Statistical differences were assessed using one-way ANOVA (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Each animal sample was measured three times, and three different animals were selected from each group. Statistical analysis was performed using nested one-way ANOVA (* $p < 0.05$). Results are presented as mean $\pm$ SD, those $n = 3$ in each experimental group B, Representative cytokine array membranes for each treatment group, showing protein spot intensities. Cytokines selected for further analysis are marked with red rectangles. C, Map of the cytokine array membrane, indicating the location of each cytokine. Analyzed proteins are highlighted in yellow.

non-fasting, basal conditions, with significant differences detected only in obese animals, suggesting that metabolic alterations in these groups may be detectable at the level of myocardial substrate preference despite the absence of corresponding normalization in systemic insulin sensitivity markers (Fig. 3.).

4.1.3. Effects of CFA and tofacitinib treatment on experimental arthritis

Hot plate tests suggest analgesic effects of tofacitinib in altered cytokine conditions like MS. Its efficacy was seen in metabolic syndrome without CFA-induced inflammation, likely through reduced pro-inflammatory cytokines [33,34]. CFA and tofacitinib both influenced paw oedema, whereas metabolic syndrome impacted this parameter only in conjunction with RA. PET scans confirmed the drug's anti-inflammatory action, CFA-induced radiotracer uptake was attenuated by tofacitinib (Additional File 1., Supplementary Figures 2., 3.) [35].

4.2. Cardiometabolic changes

4.2.1. Serum parameters related to cardiometabolic homeostasis

CK-MB and LDH levels decreased in lean animals only with

combination treatment, underscoring cytokine homeostasis's role in cardiac function control.

4.2.2. Results of cardiac imaging

Echocardiography and PET revealed that CFA and tofacitinib worsened lean animals' function but improved RA-induced dysfunction in Lean CFA Tofa rats, highlighting the differences caused by metabolic syndrome and tofacitinib treatment on cardiac function (Fig. 3., 4.). Systolic function in ZSF1 animals appeared superior to that in lean animals, possibly reflecting compensatory mechanisms leading to heart failure with preserved ejection fraction (HFpEF) [36]. E/e' confirmed worse diastolic function in ZSF1 CFA Tofa animals, consistent with histological evaluations.

4.2.3. Inflammation-induced left ventricular (LV) remodelling

Changes in IVRT, RWT, and Tei index indicate cytokine-related remodelling, with tofacitinib offering protection under high cytokine levels (Fig. 4.).

Previous studies have shown that concentric remodelling characterized by high RWT is associated with Left ventricular hypertrophy (LVH), which is an important compensatory process that develops over

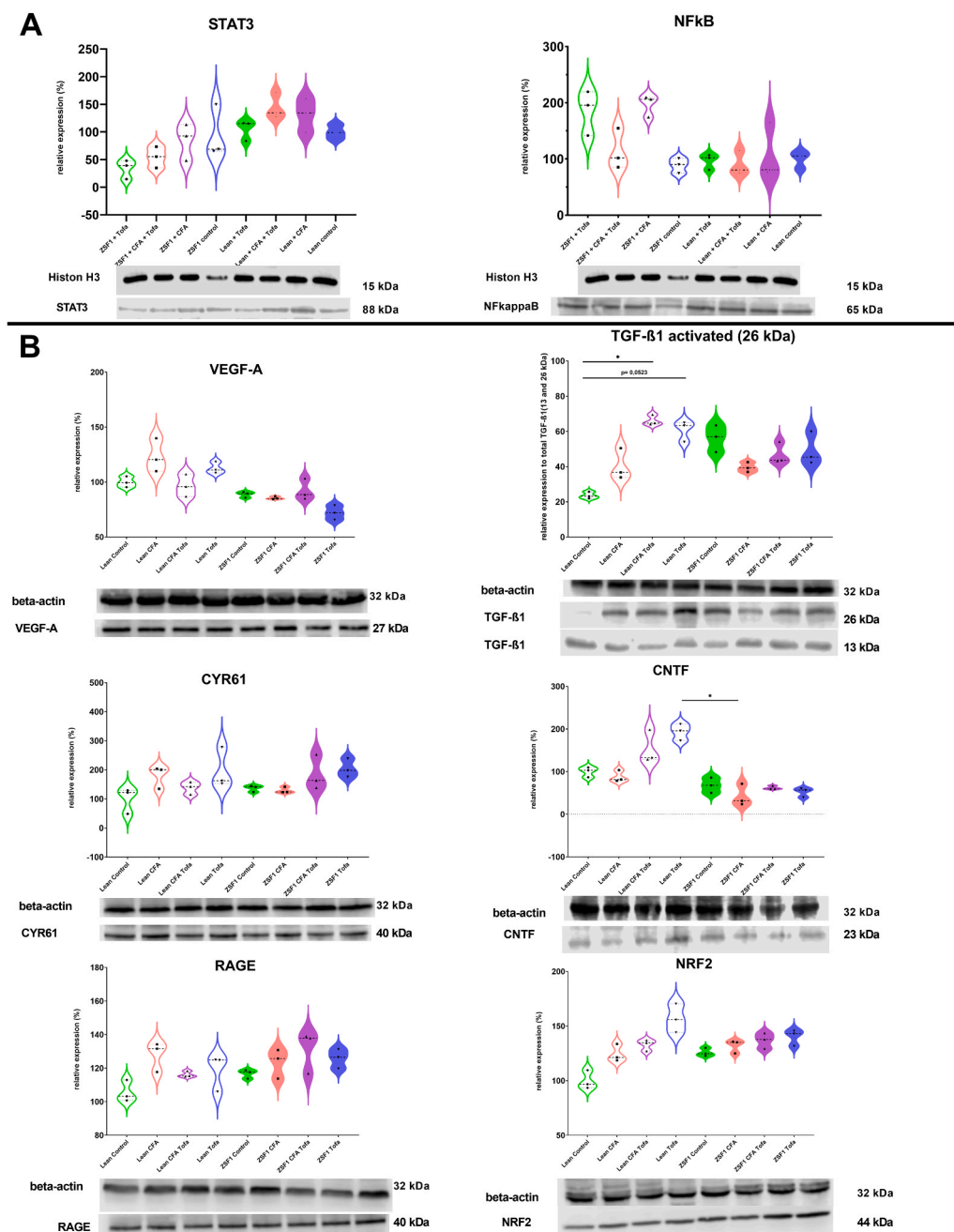


Fig. 9. Cardiac cytokine profile and protein expression in response to experimental arthritis and tofacitinib treatment examined via western blot analysis. A, Validation of selected cytokines and fibrosis-related proteins by western blot. Relative protein expression levels were quantified and normalized to β -actin. VEGF-A, RAGE, CYR61, CNTF, and NRF2 were given as relative expression in percentages, along with activated TGF- β 1 (26 kDa dimer). The relative expression of dimerized TGF- β 1 was calculated as a percentage of total TGF- β 1 [26 kDa / (26 kDa + 13 kDa) $\times$ 100]. Representative immunoblot bands are shown for each protein. Statistical analysis was performed using nested one-way ANOVA (* $p < 0.05$). Results are presented as mean $\pm$ SD. $n = 3$ in all groups B, Analysis of nuclear protein expression. Relative protein expression levels of STAT3 and NF κ B were quantified and normalized to Histone H3. Representative immunoblot bands are shown for each protein. Statistical analysis was performed using nested one-way ANOVA. Results are presented as mean $\pm$ SD. $n = 3$ in all groups.

time in response to a significant increase in diastolic ventricular pressures, often occurring in the obese population [37]. RWT was the highest in the Lean group treated with both CFA and tofacitinib, as well as in the ZSF1 group treated with CFA, which refers to inflammation-induced LV remodelling.

A heart undergoing hypertrophy typically exhibits changes in architecture and histology, where one key histopathologic component is the degree of cardiomyocyte diameter. Our observation of increased myocyte width was accompanied by a slight elevation in RWT and enhanced endothelial function. In contrast, the MS group treated with

tofacitinib did not exhibit visible hypertrophy in functional measurements (Fig. 7. B.).

4.2.4. Cytokine level changes in the cardiac tissue

Western blot analysis was employed to elucidate differences among the eight treatment groups, focusing on key cytokines implicated in CFA- and metabolic syndrome-induced inflammatory processes. The main cytokine that appears in the progression of RA, MS, and in the mechanism of action of tofacitinib is IL-6 [38,39]. Pro-inflammatory cytokines, such as IL-6 and TNF- α , are key drivers of MF [40].

In our study ZSF1 animals inoculated with CFA did not show an increase in the mass of fibrotic tissues compared to the ZSF1 control, and tofacitinib did not alter this outcome. In addition, TGF- β 1 (a principal isoform of TGF- β) is implicated in the activation of fibrosis development within cardiac tissue. The existing literature indicates that the TGF- β 1 signalling pathway facilitates the upregulation of various pro-fibrotic gene expressions such as connective tissue growth factor (CTGF) and CYR61 both being part of the CCN family [41]. In our study, lean animals showed a tendency toward increased fibrotic tissue deposition, which was accompanied by parallel activation of TGF- β 1 and CYR61, however, these changes did not reach clear statistical significance (Fig. 9. B.).

Manilall et al. reported that although IL-6 is strongly linked to adverse cardiovascular outcomes, blocking its signalling with tocilizumab did not prevent inflammation-induced left ventricular dysfunction, suggesting that other gp130-superfamily cytokines may compensate through IL-6-related pathways [42]. Consistent with this concept, elevated CNTF expression was observed in lean tofacitinib-treated animals. Although the underlying mechanism was not directly investigated, CNTF upregulation may represent a compensatory response to JAK/STAT pathway inhibition and could contribute to the cardiac remodelling pattern observed in this group [43–45]. The observed interaction between CNTF and TGF- β further underscores the complex role of cytokine balance in regulating cardiac remodelling across different conditions (Fig. 10.).

4.2.5. Emerging interrelations

In lean animals, CFA immunization induced mild experimental autoimmune myocarditis (EAM) via IL-6-mediated inflammation [46], while in ZSF1 rats, fibrosis progression was aggravated by hypertension, hyperglycaemia, insulin resistance, and metabolic dysfunction.

Myocardial fibrosis is a key component of LVH and contributes to progressive diastolic dysfunction in both inflammatory and metabolic cardiac disease. Persistently elevated wall stress and compromised myocardial perfusion led to myocyte degradation and extracellular matrix accumulation. In the present study, Masson's trichrome staining confirmed fibrotic deposition predominantly in obese animals, whereas lean groups showed only minimal changes [47] (Fig. 7. C.). Cardiac remodelling in this model is driven by overlapping inflammatory and metabolic stressors, including Ang II-mediated pressure overload, AGE-RAGE activation under hyperglycaemic conditions, and JAK/STAT-associated pathways [48,49] (Fig. 10.).

EAM with MS comorbidities intensified cardiac hypertrophy and

diastolic dysfunction, though fibrosis remained stable, whereas lean tofacitinib-treated rats showed mild fibrotic remodelling despite preserved cardiac function. In this context, JAK inhibition alone was insufficient to attenuate established fibrosis. Lean tofacitinib-only animals exhibited subtle fibrotic remodelling while maintaining preserved global cardiac function, suggesting a potential drug-related or compensatory effect [50], although direct evidence for activation of the Ang II-JAK/STAT axis was not obtained in the present study.

Compensatory mechanisms included pathological arterial relaxation, reduced diastolic blood pressure, increased heart rate, and enhanced glucose uptake on [18 F]FDG-PET, alongside enlarged cardiomyocytes and elevated CNTF (Fig. 9.). As both lean and ZSF1 rats received tofacitinib in the absence of autoimmune induction, the findings suggest potential adverse cardiac remodelling under non-inflammatory conditions, consistent with safety concerns reported for JAK inhibitors in diseases lacking systemic inflammation, such as alopecia areata [51].

5. Conclusion

Our study revealed that while tofacitinib effectively improved cardiac function and metabolic parameters in the context of comorbid RA and MS, it also produced unexpected adverse cardiac effects under non-inflammatory conditions. These findings highlight the central yet complex role of the JAK/STAT pathway in cardiac remodelling, beneficial for inflammation-driven dysfunction but insufficient to prevent fibrosis. Tofacitinib showed favorable effects on blood pressure, lipid, and glucose metabolism in MS, yet may be associated with signs of adverse cardiac remodelling under non-inflammatory conditions. Although several cytokine alterations (including TGF- β 1, CYR61, and CNTF) were associated with the observed structural and functional cardiac changes and with markers suggestive of early fibrotic remodelling, the present study was not designed to establish causal mechanistic links between JAK inhibition and fibrosis development. Consequently, the proposed mechanisms should be interpreted as biologically plausible hypotheses supported by current findings, warranting further investigation in dedicated mechanistic studies. Overall, the distinct cytokine-driven mechanisms underlying RA and MS underscore the need for carefully tailored and possibly combination-based JAK-targeted therapies, accounting for metabolic status to maximize efficacy and minimize harm (Fig. 10.).

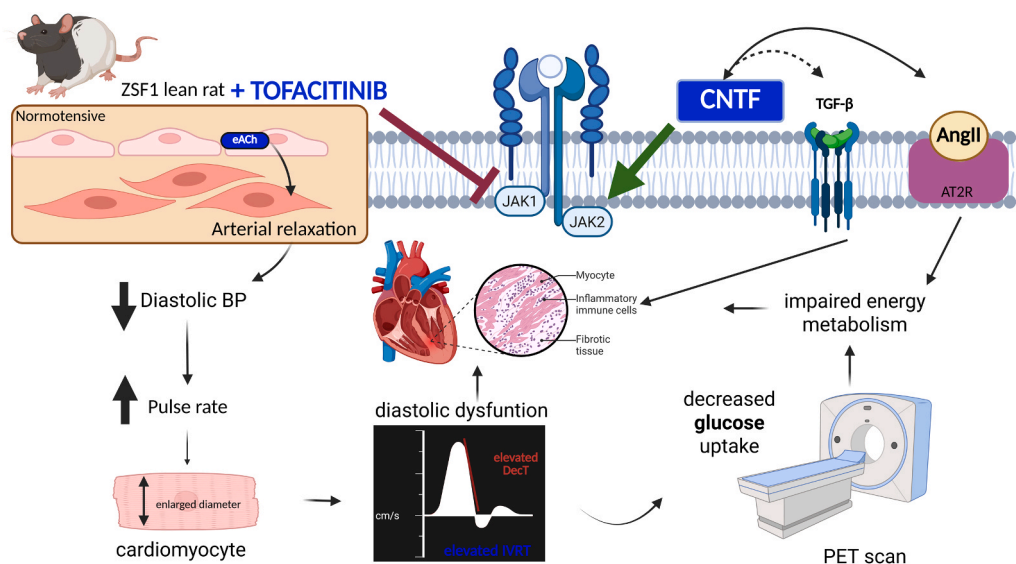


Fig. 10. Graphical evaluation of the section Conclusion.

Limitations of the study

The relatively short study duration and limited sample size, restricted to male animals, represent important limitations of this work. A further limitation is the unequal number of animals included across the different experimental analyses, which arose due to technical constraints inherent to complex in vivo and ex vivo sample processing. Another limitation of the present study is the translational gap between the experimental models and human disease. In particular, while the ZSF1 rat model and CFA-induced arthritis model recapitulate key features of cardiometabolic dysfunction and systemic inflammation, respectively, they do not fully reflect the complexity and chronic progression of human rheumatoid arthritis and its associated cardiovascular comorbidities. Therefore, caution is warranted when extrapolating the present findings to clinical settings.

CRedit authorship contribution statement

Panna Görögh: Visualization, Project administration, Methodology, Investigation. **Rita Kiss:** Validation, Resources, Project administration, Methodology, Funding acquisition. **Mariann Bombicz:** Writing – original draft, Visualization, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Gábor Viczján:** Project administration, Methodology, Investigation, Data curation. **Tamás Erdei:** Project administration, Methodology, Investigation, Data curation. **Botond Gaál:** Methodology, Investigation, Data curation, Conceptualization. **Reka Szekeres:** Project administration, Methodology, Investigation, Data curation. **György Trencsenyi:** Validation, Project administration, Methodology, Investigation. **Barbara Takács:** Project administration, Methodology, Investigation, Data curation. **Tymur Vietrov:** Project administration, Methodology, Investigation, Data curation. **Vera Tarjányi:** Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Michelle Adeagbo:** Project administration, Methodology, Investigation, Data curation. **Anna Szilágyi:** Writing – review & editing, Methodology, Investigation, Data curation. **Béla Juhász:** Writing – review & editing, Validation, Resources, Funding acquisition, Conceptualization. **Zoltán Szilvassy:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Rudolf Gesztelyi:** Software, Methodology, Investigation, Formal analysis, Data curation. **Attila Hamar:** Validation, Project administration, Investigation, Conceptualization. **Balázs Varga:** Project administration, Methodology, Investigation, Data curation. **Dániel Priksz:** Visualization, Methodology, Formal analysis, Data curation, Conceptualization.

Ethics approval and consent to participate

The studies were approved by the Ethics Committee of the University of Debrecen, and the humane care of the animals matched the expectations of the “Principles of Laboratory Animal Care” by EU Directive 2010/63/EU (license no. 7/2020/DEMÁB).

Consent for publication

Not applicable.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT (OpenAI; model version GPT–5.2) in order to suggest edits to grammar, sentence structure, and wording. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vera Tarjányi, Anna Szilágyi, Reka Szekeres, Barbara Takacs, Balázs Varga, Daniel Priksz, Rudolf Gesztelyi, Gabor Viczjan, Tamas Erdei, Panna Gorogh, Rita Kiss, Zoltan Szilvassy, Bela Juhasz reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund. Mariann Bombicz reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund. Vera Tarjányi, Anna Szilágyi, Reka Szekeres, Barbara Takacs, Balázs Varga, Daniel Priksz, Rudolf Gesztelyi, Gabor Viczjan, Tamas Erdei, Rita Kiss, Gyorgy Trencsenyi, Zoltan Szilvassy, Bela Juhasz reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by European Union and the European Regional Development Fund. Mariann Bombicz reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by European Union and the European Regional Development Fund. Anna Szilágyi, Balázs Varga, Daniel Priksz, Rudolf Gesztelyi, Gabor Viczjan, Tamas Erdei, Rita Kiss, Zoltan Szilvassy, Bela Juhasz reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by Ministry of Innovation and Technology in Hungary. Vera Tarjányi reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by Therapeutic Purpose Development thematic programme of the University of Debrecen. Mariann Bombicz reports financial support, article publishing charges, equipment, drugs, or supplies, and travel were provided by Ministry of Innovation and Technology in Hungary. Vera Tarjányi, Anna Szilágyi, Reka Szekeres, Barbara Takacs, Balázs Varga, Daniel Priksz, Rudolf Gesztelyi, Gabor Viczjan, Tamas Erdei, Panna Gorogh, Rita Kiss, Zoltan Szilvassy, Bela Juhasz reports a relationship with Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund (TKP2021-EGA–18) that includes: funding grants. Mariann Bombicz reports a relationship with Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund (TKP2021-EGA–18) that includes: funding grants. Vera Tarjányi, Anna Szilágyi, Reka Szekeres, Barbara Takacs, Balázs Varga, Daniel Priksz, Rudolf Gesztelyi, Gabor Viczjan, Tamas Erdei, Rita Kiss, Gyorgy Trencsenyi, Zoltan Szilvassy, Bela Juhasz reports a relationship with European Union and the European Regional Development Fund (GINOP –2.3.4–15–2016–00002) that includes: funding grants. Mariann Bombicz reports a relationship with European Union and the European Regional Development Fund (GINOP –2.3.4–15–2016–00002) that includes: funding grants. Anna Szilágyi, Balázs Varga, Daniel Priksz, Rudolf Gesztelyi, Gabor Viczjan, Tamas Erdei, Rita Kiss, Zoltan Szilvassy, Bela Juhasz reports a relationship with Higher Education Institutional Excellence Programme of the Ministry of Innovation and Technology in Hungary that includes: funding grants. Mariann Bombicz reports a relationship with Higher Education Institutional Excellence Programme of the Ministry of Innovation and Technology in Hungary that includes: funding grants. Vera Tarjányi reports a relationship with Therapeutic Purpose Development thematic programme of the University of Debrecen that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.biopha.2026.119772](https://doi.org/10.1016/j.biopha.2026.119772).

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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