Research Square

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SGLT2 inhibition attenuates γδ T cell–mediated meta-inflammation associated with cardiometabolic risk in type 2 diabetes

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Research Article

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Abstract

Background

Cardiovascular and renal protection conferred by sodium–glucose cotransporter-2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1RA) extends beyond glucose lowering. Chronic low-grade meta-inflammation is a key driver of cardiometabolic risk in type 2 diabetes (T2D), yet the cellular mediators modified by modern antidiabetic therapy remain poorly defined. We investigated whether SGLT2i alone or combined with GLP-1RA attenuate γδ T cell mediated meta-inflammation in T2D.

Methods

Twenty-five patients with poorly controlled T2D initiating SGLT2i therapy (n = 15) or SGLT2i plus GLP-1RA (n = 10) were prospectively followed for 12 months. Age- and sex-matched non-diabetic controls (n = 30) were included at baseline. Peripheral blood γδ T cell phenotype and cytokine production were assessed by multiparametric flow cytometry. Metabolic, hepatic, and renal parameters were evaluated longitudinally.

Results

At baseline, T2D was associated with increased interferon-γ (IFN-γ) production by both Vδ1$^+$ and Vδ2$^+$ γδ T cells without changes in subset frequency. Antidiabetic treatment induced a sustained reduction in IFN-γ production of up to 40% at 12 months ($p < 0.01$), independent of glycemic improvement. Decreases in IFN-γ correlated with improved insulin sensitivity (HOMA-IR) and reduced fat mass, but not with HbA1c reduction. The anti-inflammatory effect was most pronounced in patients with mild hepatic or renal impairment and correlated with liver fibrosis indices and renal function markers.

Conclusions

Modern antidiabetic therapy attenuates γδ T cell-mediated meta-inflammation independently of glucose lowering. Reduction of IFN-γ production associates with improved insulin resistance and early organ involvement, identifying γδ T cells as a potential immuno-metabolic biomarker linking metabolic therapy to cardiovascular and renal protection.

Research insights

What is currently known about this topic?

SGLT2 inhibitors and GLP1 mimetics give cardiovascular protection early after start of treatment. Meta-inflammation is a cardiovascular risk factor. γδ T cells are causally linked to metabolic disease

What is the key research question?

How does modern anti-diabetic therapy impact meta-inflammation mediated by γδ T cells?

What is new?

SGLT2 inhibition alone or in combination with GLP1 mimetics strongly reduce IFNγ production by γδ T cells. Amelioration of meta-inflammation correlated with insulin resistance independent of glycemic control

How might this study influence clinical practice?

γδ T cell mediated meta-inflammation may be an independent biomarker of cardiometabolic risk

Introduction

In recent years, the scope of antidiabetic treatment has shifted from a narrow focus on glucose control to a more comprehensive approach that includes additional health benefits such as weight management and the reduction of cardiorenal instead of cardiovascular risk [1, 2]. The ADVANCE and UKPDS trials have shown that levels of glycated hemoglobin A_{1c} (HbA_{1c}) positively correlate with the risk of microvascular disease, stroke, heart failure incidence and myocardial infarction [3, 4]. In addition, a 1% reduction in HbA_{1c} wash shown to translate to a 14% decrease in all-cause mortality among patients with type 2 diabetes mellitus (T2D), coupled with a notable 21% reduction in diabetes-related mortality [4]. Modern antidiabetic drugs, such as sodium-glucose cotransporter-2 (SGLT2) inhibitors and glucagon-like peptide-1 receptor agonists (GLP-1RAs), have been shown to be particularly potent in reducing health risks in people with type 2 diabetes mellitus (T2D). Core clinical trials have demonstrated the efficacy of SGLT2i in reducing major adverse cardiovascular events, hospitalizations for heart failure and slowing the progression of chronic kidney disease. Similarly, GLP-1RAs enhance glucose-dependent insulin secretion and have shown significant cardiovascular benefits [5, 6].

Interestingly, the beneficial effects of modern antidiabetic drugs appear to depend on other factors than regulation of glycemia alone. Head-to-head comparative studies consistently show that SGLT2 inhibitors and GLP-1 receptor agonists are associated with lower rates of diabetic complications such as major adverse cardiovascular events (MACE) and heart failure hospitalization than older antidiabetic drugs such as DPP-4 inhibitors and sulfonylureas in routine clinical practice [7]. These advantages persist after adjustment for baseline HbA_{1c} and other cardiometabolic risk factors, and for SGLT2 inhibitors, remain remarkably consistent across categories of glycemic control [8, 9]. Together with cardiovascular and renal outcome trials demonstrating benefits in individuals without diabetes, such as the EMPEROR-preserved and SELECT trials [10, 11], these data support the notion that SGLT2 inhibitors and GLP-1 receptor agonists confer cardiovascular protection that is only partly explained by glucose lowering.

One possible mechanism via which modern antidiabetic drugs mediate their beneficial effect is through control of inflammation. Metabolic disease is associated with chronic systemic inflammation, also referred to as meta-inflammation, due to tissue stress in organs such as liver and adipose tissue, which accumulate large amounts of nutrients [12]. Large prospective cohorts study including over 20 000 participants showed that elevated inflammatory markers such as high-sensitivity C-reactive protein (hsCRP) and interleukin (IL) 6 in circulation predict incident myocardial infarction, stroke, and cardiovascular mortality even in otherwise healthy individuals, outperforming several traditional risk factors [13]. In patients with diabetes, higher CRP levels were an independent risk factor for the development of MACE [14]. Of note, treatment of patients with SGLT2 inhibitors or GLP-1RAs resulted in a reduction of inflammatory markers [15, 16]. Unfortunately, serum levels of molecules such as CRP and IL-6 are typically low, requiring assays of very high sensitivity and large patient groups to show differences. Moreover, these markers are associated with general inflammation and are not specific for the underlying pathophysiology of T2D. This precludes their use as a biomarker for disease status and therapy responsiveness. Thus, a more specific link between inflammation, diabetes and its treatment may therefore be beneficial for better diagnosis and efficiency of treatment.

Previously, our team found that cytokine production of specific immune cells was increased in patients with uncontrolled T2D compared to controls [17]. In particular, interferon (IFN) γ production by blood γδ T cells was increased. IFNγ is a cytokine which has been causally linked with blood glucose and lipid regulation in animal models [18–20]. Here, we investigated whether antidiabetic treatment with SGLT2 inhibitors alone, or in combination with GLP-1RA ameliorated immune cell hyper-reactivity. We observed that both for δ1 and δ2 γδ T cells IFNγ production significantly decreased for at least 12 months after initiation of treatment. Reduction of hyperreactivity positively correlated with insulin sensitivity and fat mass. Our findings provide a valuable new link between T2D and systemic inflammation with great potential as a future biomarker for therapy efficiency.

Methods

Subjects

Adult patients with T2D eligible for antidiabetic treatment modification, including SGLT2 inhibitors and/or GLP-1 receptor agonists as add-on therapy to metformin, were recruited from the Centre of Diabetes, Endocrinology and Cardiometabolism, the diabetes outpatient clinic at the Special Hospital Thalassotherapia Opatija, Croatia, between November 2020 and October 2023. The diagnosis of T2D was confirmed based on the latest American Diabetes Association (ADA) guidelines [21].

The presence of exclusion criteria was established through medical history, clinical examination, and initial laboratory evaluation, omitting patients with the following immune-modulating factors: diagnosis of type 1 diabetes mellitus or latent autoimmune diabetes in adults (LADA; indicated by decreased C-peptide levels or positive autoantibodies to glutamic acid decarboxylase or islet antigen 2), acute infection or recent vaccination (within the past 4 weeks), recurrent urinary tract infections, chronic

inflammatory diseases, active malignant diseases, disorders affecting the hypothalamus-pituitary-adrenal axis, use of anti-inflammatory or immunosuppressive medications, advanced liver or kidney disease. Additionally, patients taking insulin, or more than two oral antidiabetic drugs were not included in the study. Participants who fulfilled all exclusion criteria but did not meet the criteria for diabetes diagnosis [21] were included in the control group (**Table S1**).

Patients with uncontrolled type 2 diabetes were treated with SGL2 inhibitor (empagliflozin or dapagliflozin, administered once daily) either as monotherapy or in combination with GLP-1 receptor agonist semaglutide (administered once weekly by subcutaneous injection), in addition to a maximally tolerated dose of metformin. If participants reached the target HbA_{1c} value of < 7.0% after 3 months of modified antidiabetic treatment, blood samples were collected again at the 6-month mark. If the glycemic target was not achieved after 3 months, the treatment regimen or dosage was adjusted, and the follow-up period was extended to 9 months according to the study diagram (Study Flowchart). If the glycemic target was not met 6 months after the antidiabetic treatment modification, if treatment was prematurely discontinued due to adverse effects, or if the patient was lost to follow-up, blood collection was not repeated at the 6-month point and the patient was excluded from the analysis.

Ethical considerations

The ethics committee of the Institutional Review Board at Thalassotherapia Opatija approved this research under number 01-000-00-503/2-2020. We conducted the research in accordance and agreement with the International Conference on Harmonization Good Clinical Practice Guidelines and with the Declaration of Helsinki. All participants were over 18 years old and provided informed consent prior to inclusion in the study.

Blood isolation

Peripheral venous blood was collected between 7 and 9 a.m. in ethylenediaminetetraacetic acid (EDTA) and serum gel activator tubes from individuals who had fasted for at least 10 hours. Antidiabetic medications were discontinued 48 hours before the blood collection. Hematological and biochemical analysis were conducted using standard methods. Serum was tested for autoantibodies to exclude patients with LADA. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, St. Louis, Missouri, United States) or Lymphoprep (ProteoGenix, Schiltigheim, France) within 6–8 hours of blood collection. After isolation, the cells were cryopreserved in foetal bovine serum (Corning, Inc., Corning, New York, United States) and 10% dimethyl sulfoxide (Sigma-Aldrich, St. Louis, Missouri, United States) until use.

Flow cytometry

Flow cytometric and intracellular flow cytometric analyses was performed on thawed samples according to standard protocols. Briefly, cells were washed and stained with fluorochrome-conjugated antibodies for specific surface markers for 30 minutes at 4°C in the dark. For intracellular staining, cells were first

stained for surface markers for 30 minutes at 4°C, followed by permeabilization and fixation using either the intracellular fixation and permeabilization set or the Foxp3 transcription factor fixation and permeabilization set (eBioscience, Inc., San Diego, California, United States). Intracellular molecules were then stained for 20 minutes at room temperature using anti-human antibodies. All antibodies used in the study are summarized in Supplementary Table S2. Data were acquired using a BD FACSAriaIIu flow cytometer (BD, Franklin Lakes, New Jersey, United States) and analyzed with FlowJo software (FlowJo LLC, Ashland, Oregon, United States). The gating strategy followed the Guidelines for the use of flow cytometry and cell sorting in immunological studies [22].

In vitro stimulations

Upon thawing, isolated PBMCs were stained with a 0.4% trypan blue solution (ThermoFisher Scientific, Waltham, Massachusetts, United States), enumerated, and assessed for viability using CytoSMART (CytoSMART Technologies, Eindhoven, Netherlands). To measure cytokine production, cells were incubated at 37°C with 5% CO2 in the presence of 20 ng/ml phorbol-12-myristate-13-acetate (PMA, Sigma-Aldrich, St. Louis, Missouri, United States) for 4 hours in the presence of Brefeldin A (eBioscience, San Diego, California, United States).

Anthropometric measurements and body composition analysis

Anthropometric measurements included body weight, height, waist and hip circumference. Weight and height were measured using a high-precision digital column scale with a stadiometer (Seca, Hamburg, Germany). A standard cloth tape measure was used to record waist and hip circumference. Body composition was estimated using bioelectrical impedance analysis technology (InBody, Seoul, South Korea).

Statistical analysis

Data are presented as paired individual values. Depending on the type of variables, normality of data distribution, and sample characteristics, statistical significance was determined by Fisher's exact test, Chi-square test, Mann-Whitney test, Wilcoxon test, and Kruskal-Wallis test followed by Dunn's post-hoc test. Correlation analysis was used to determine the Spearman coefficient. A p-value < 0.050 was considered statistically significant, with specific significance levels reported in the figure captions. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, California, United States).

Results

Antidiabetic treatment ameliorates IFNγ production by γδ T cells

Previously, we have shown that T2D does not impact the frequency of γδ T cells in the blood of patients, but causes an increase in the production of cytokines [17]. Indeed, prior to treatment, we observed that T-

cell receptor (TCR) $\delta 1^+$ γδ T cells isolated from the blood of patients with T2D expressed higher levels of IFNγ then cells of matched controls upon in vitro restimulation with PMA and ionomycin (**Figure 1a, Table S3**). Patients were segregated into two groups, one treated with metformin and SGLT2 inhibitors alone (SGLT2i) or in combination with GLP1-R agonists (**Study Flowchart**). As expected, apart from the improvement of glycemic parameters, which were used as a selection criterium for effective treatment, we observed significant improvement of the serum lipid profile of patients. In addition, we observed improvement in liver parameters, including reduced aspartate aminotransferase (AST), alanine aminotransferase (ALT) and hepatic steatosis index, whereas renal function was not significantly impacted (**Table 1**).

Next, we investigated whether antidiabetic treatment had an impact on meta-inflammation mediated by γδ T cells. PBMCs were isolated from patients at time of initial visit, after 6 months of treatment and after 12 months of treatment. Cells from each timepoint were stimulated

Table 1

Characteristics of patients at baseline and after 12 months of antidiabetic treatment. eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein.

Age, years	**Baseline (*n* = 25)**	**12 months (*n* = 25)**	***p* value**
	60 ± 8 (44–74)		
Female sex, n (%)	10 (40)		
Glycated haemoglobin A_{1c}, %	8.3 ± 1.1	6.6 ± 0.4	< 0.001
Fasting plasma glucose, mmol/l	9.3 ± 2.1	6.9 ± 0.8	< 0.001
HOMA-insulin resistance	7.9 ± 4.5	4.5 ± 3.3	< 0.001
HOMA-beta cell function	7.5 ± 4.1	8.1 ± 4.9	0.465
Quantitative insulin sensitivity check index	0.29 ± 0.02	0.32 ± 0.03	< 0.001
Body mass index, kg/m^2	30.7 ± 4.1	28.4 ± 3.7	< 0.001
Body fat, %	29.8 ± 8.7	29.5 ± 8.4	0.733
Skeletal muscle index, kg/m^2	8.8 ± 1.3	8.2 ± 1.1	< 0.001
Waist-hip ratio	0.97 ± 0.07	0.97 ± 0.05	0.565
Leukocyte count, $\times 10^9$ per litre	7.7 ± 2.2	8.3 ± 2.1	0.138
C reactive protein, mg/l	2.9 ± 2.4	1.9 ± 1.9	0.114
Triglycerides, mmol/l	1.8 ± 1.2	1.6 ± 0.9	0.111
HDL cholesterol, mmol/l	1.2 ± 0.4	1.3 ± 0.3	< 0.001
LDL cholesterol, mmol/l	2.8 ± 1.0	2.2 ± 1.1	0.030
Total cholesterol, mmol/l	4.6 ± 1.3	4.0 ± 1.3	0.024
Statin use, *n* (%)	13 (52)	23 (92)	
Aspartate aminotransferase, U/l	29.2 ± 13.5	27 ± 12	0.028
Alanine aminotransferase, U/l	29.3 ± 22.4	29 ± 13	< 0.001
Hepatic steatosis index	44.1 ± 6.1	39.9 ± 4.8	< 0.001
Triglyceride-glucose index	9.3 ± 0.6	8.9 ± 0.6	< 0.001
NAFLD-liver fat score	2.5 ± 1.8	1.4 ± 1.6	0.002
Urea, mmol/l	5.9 ± 1.3	6.5 ± 1.9	0.096
Creatinine, µmol/l	76.1 ± 13.4	75.0 ± 17.9	0.595

Age, years	Baseline ($n = 25$)	12 months ($n = 25$)	p value
	60 ± 8 (44–74)		
eGFR, ml/min/1.73m^2	83.4 ± 15.7	87.0 ± 17.5	0.090

simultaneously and their phenotype and function were analysed. In the blood, most γδ T cells express either the TCR δ1 or δ2 chain, with the latter type typically being prevalent approximately five times more. δ1$^+$ T cells are thought to predominantly produce cytokines, such as IFNγ, tumor necrosis factor (TNF) and IL-17A, whereas δ2$^+$ cells are considered to have higher cytotoxic potential and express more Granzyme B. We first analysed δ1$^+$ γδ T cells. Antidiabetic treatment did not impact the frequency of these cells as a fraction of total CD3$^+$ T cells (Fig. 1b). Expression of the costimulatory molecules CD27 and CD28 has been associated with the ability to produce specific cytokines such as IL-17A, both in humans and mice [23]. However, no changes were observed upon antidiabetic treatment. CD16, a protein associated with antibody-dependent cytotoxicity [24] was also present in

Figure 1, Antidiabetic treatment ameliorates IFNγ production by δ $_1$ $^+$ γδ T cells. *(a) Peripheral blood mononuclear cells from patients with type 2 diabetes mellitus and matched controls were stimulated and interferon (IFN) γ production by δ_1^+ γδ T cells was determined using intracellular flow cytometry at baseline. (b) δ_1^+ γδ T cells as a frequency of total CD3$^+$ T cells after antidiabetic treatment. (c) Tumor necrosis factor (TNF) and interleukin (IL) 17A production by peripheral blood δ_1^+ γδ T cells upon in vitro restimulation after indicated times of antidiabetic treatment. (d) IFNγ production by peripheral blood δ_1^+ γδ T cells upon in vitro restimulation after indicated times of antidiabetic treatment. Shown are representative fluorescence activated cell sorting plots of CD45$^+$CD3$^+$$\delta_1^+$ cells from the same patient over time. An individual point represents one participant. Indicated are mean values ± standard error* of mean and statistical significances at *$p < 0.050$ and **<0.010 by (a) Mann-Whitney and (d) one-way ANOVA with Tukey's multiple comparisons post testing.

equal amount on the surface of δ1$^+$ γδ T cells at all time points after initiation of treatment (**Figure S2b**). Finally, expression of CD45RA, as a marker of terminal effector differentiation, was also not affected by any of the time points (**Figure S2c**). Thus, antidiabetic treatment did not appear to impact the phenotype of δ1$^+$ γδ T cells.

To determine whether antidiabetic treatment impacts immune cell responsiveness, cells were restimulated in vitro using PMA and ionomycin and cytokine production was assessed by intracellular flow cytometry. Antidiabetic treatment did not impact the ability of δ1$^+$ γδ T cells to produce either TNF or IL-17A. In contrast, we observed a strong impact on the production of IFNγ. At six months of treatment, we observed an absolute reduction of 11% (± 26.3) and a relative reduction of 20% (± 48.0). After 12 months, the absolute reduction was 17.2% (± 17.1%) whereas the relative reduction was 39.2% (± 31.2), which reached statistical significance at this point.

Subsequently, we investigated how antidiabetic treatment impacts δ2$^+$ γδ T cells. Neither at 6, nor at 12 months of treatment did we observe changes in the frequency of these cells as a fraction of CD3$^+$ T cells (Fig. 2a). Phenotypic analysis showed a minor shift of a more active CD28$^+$CD27$^+$ to a more anergic CD28$^-$CD27$^-$ phenotype (**Figure S2d**). This corresponded with a minor increase in expression of CD45RA at 12 months of treatment. Expression of CD16 was not affected on these cells at any time point (**Figure S2e,f**).

Similar to δ1$^+$ γδ T cells, we did not observe changes in the ability of δ2$^+$ γδ T cells to produce TNF. As expected, these cells secrete very little IL-17A, which was not impacted by antidiabetic treatment. Instead, these cells produce Granzyme B, which was also not affected. Again, we observed a strong impact of antidiabetic treatment on production of IFNγ. Already after 6 months we observed a significant reduction in the production of this cytokine, with an absolute reduction of 14.1% (± 25.1) and a relative reduction 25.1% (± 44.8). This reduction was slightly increased at 12 months, with an absolute reduction of 17.4% (± 16.5) and a relative reduction of 41.5% (± 29.5).

Thus, antidiabetic treatment does not impact the frequency of γδ T cells and only has a mild impact on their phenotype. In contrast, antidiabetic treatment ameliorates the hyperresponsiveness of γδ T cells, with a specific impact on IFNγ production.

GLP1R agonists do not further enhance the anti-inflammatory effect of SGLT2 inhibition

Both SGLT2 inhibitors and GLP-1R agonists have a broad, beneficial impact on metabolic parameters in patients with T2D. To investigate whether they have a compound effect, we segregated these groups based on whether they received SGLT2 inhibitors only or in combination with GLP-1R agonists and studied cytokine production. Segregation in subgroups did not reveal an independent effect of antidiabetic drugs on the production of TNF or IL-17A by δ1$^+$ γδ T cells (**Figure S4a,b**). Neither did it uncover and effect on production of TNF and Granzyme B by δ2$^+$ γδ T cells.

We then focused on IFNγ. Patients treated with SGLT2 inhibitors on average showed a 20% (± 18.4) reduction in the frequency of δ1$^+$ γδ T cells producing IFNγ upon in vitro restimulation (Fig. 3a). Add-on treatment with incretin mimetics did not cause a further reduction in the secretion of IFNγ (12.9%±13.9). Indeed, the relative reduction in IFNγ production was not significantly different between treatment groups (33.4%±33.3 vs. 32.0%±34.5, Fig. 3b).

Comparable observations were made for δ2$^+$ γδ T cells. The group treated with SGLT2 inhibitors only showed an absolute reduction in IFNγ production of 18.7% (± 19.4) versus 15.5% (± 10.5) in the group receiving add-on GLP1R agonists (Fig. 3c). This resulted in a relative reduction of 29.0% (± 32.2) and 32.7% (± 23.8) in patients treated with SGLT2i alone or in combination with GLP1R agonists, which was not significantly different between these two groups (Fig. 3d).

In summary, treatment of patients with SGLT2 inhibitors results in a significant reduction of γδ T cell hyperresponsiveness, which is not further ameliorated by GLP1 receptor agonist add-on therapy.

IFNγ levels correlate with changes in HOMA-IR and Fat mass

We subsequently wanted to elucidate which metabolic parameters may be associated with changes in the inflammatory status of γδ T cells. We therefore performed a post hoc analysis to identify correlations between IFNγ production and physiological parameters associated with metabolic syndrome. Strikingly, changes in IFNγ did not correlate with relative alterations in any metabolic parameter investigated (Fig. 4a). We therefore first wanted to explore in which patient subgroup alterations of IFNγ were most prominent. We correlated changes in IFNγ with absolute levels of metabolic parameters. Advanced liver and kidney pathology were exclusion criteria for this study. Nevertheless, we observed that changes in the levels of this cytokine correlated with serum levels of AST and with the FIB4 fibrosis index, indicative of mild liver damage. This was the case both for $\delta1^+$ and $\delta2^+$ γδ T cells (Fig. 4b). Importantly, for $\delta2^+$ γδ T cells, changes in IFNγ also correlated with increased urea and creatinine levels, which may be indicators of chronic kidney disease. Notably, changes in IFNγ expression also correlated with liver and kidney pathology prior to treatment, both for $\delta1^+$ and $\delta2^+$ γδ T cells (**Figure S4a,b**), as indicated by a positive correlation with FIB4 and serum urea levels.

To gain insight in the potential impact of IFNγ produced by γδ T cells on metabolic syndrome, we correlated absolute expression levels of this cytokine with changes in the values of metabolic parameters. We observed that IFNγ levels positively correlated with changes in the HOMA-IR index, as well as with changes in fat mass. We observed this both for $\delta1^+$ and $\delta2^+$ γδ T cells (Fig. 4c).

Thus, changes in IFNγ production by γδ T cells were most prominent in patients with mild pre-existing liver and kidney damage. Moreover, the level of IFNγ expression by these cells corresponded with adipose tissue mass, as well as insulin resistance, but not with other parameters of metabolic syndrome.

Discussion

Previous studies have examined the effects of modern antidiabetic therapies on systemic markers of inflammation [25, 26]. However, their impact on immune cell populations directly implicated in metabolic pathology has remained largely unexplored. Here, we demonstrate that treatment with SGLT2 inhibitors alone, or in combination with GLP-1 receptor agonists leads to a marked reduction in IFN-γ production by γδ T cells. Importantly, this reduction in γδ T cell–mediated meta-inflammation was closely associated with improved insulin sensitivity and a decrease in fat mass, identifying IFN-γ production by these cells as a potential biomarker of therapeutic responsiveness in type 2 diabetes.

Both SGLT2 inhibitors and GLP-1 receptor agonists are known to reduce cardiovascular risk, even in individuals without established type 2 diabetes, and these benefits emerge rapidly after treatment initiation [10, 27]. Chronic inflammation is a recognized driver of cardiovascular disease. However, antidiabetic therapies exert only modest effects on conventional inflammatory markers such as CRP and

IL-6 [25, 26]. This likely reflects the fact that within the immunological response, these proteins are primarily associated with acute inflammatory responses, particularly following bacterial infection, and are only weakly induced by the chronic, low-grade inflammatory processes characteristic of metabolic disease [28, 29]. Accordingly, detection of treatment-induced changes in these markers often requires high-sensitivity assays [30]. In contrast, we and others have previously shown in experimental models that metabolic stress in organs such as liver and adipose tissue is sensed by innate-like lymphocytes, including natural killer cells and γδ T cells [31, 32]. IFN-γ produced by these cells promotes local inflammation and directly contributes to insulin resistance [19]. Consistent with this, we observed up to a 40% reduction in IFN-γ production by γδ T cells following treatment, which correlated with reduced fat mass and improved insulin sensitivity. The absence of a comparable effect on tumor necrosis factor production may reflect the predominant role of macrophages, rather than γδ T cells, as the main source of this cytokine in metabolic disease [33]. Together, these findings support IFN-γ production by γδ T cells as a sensitive and patho-physiologically relevant readout of meta-inflammatory improvement.

Both SGLT2 inhibitors and GLP1 receptor agonists have previously been reported to ameliorate meta-inflammation in patients with type 2 diabetes [15, 25]. Surprisingly, combination therapy was not associated with greater improvements in systemic inflammatory markers compared with monotherapy, even though to date this has not been a primary outcome of any comparative study [34]. Accordingly, we did not observe a further reduction in IFN-γ production in patients receiving combined SGLT2 inhibitor and GLP-1 receptor agonist therapy compared with those treated with SGLT2 inhibition alone. One possible explanation is that γδ T cell–mediated meta-inflammation primarily reflects the absolute metabolic state of affected tissues, particularly adipose tissue, rather than relative changes in metabolic parameters [35]. Although patients receiving combination therapy showed larger relative improvements with regards to metabolic parameters, they also presented with more severe disease at baseline. Following treatment, absolute measures such as glycemia and body mass index were comparable between groups (**Table S4**). These findings suggest that attenuation of γδ T cell–driven meta-inflammation depends on restoration of tissue metabolic homeostasis, rather than on the magnitude of metabolic improvement per se.

Limitations of the study

Limitations of this study include the narrow scope of investigated cells. Whereas γδ T cells have been associated with the underlying pathophysiology of T2D, other immune cells are also involved and have not been studied here. Also, our study has a relatively small number of participants, which may impact our ability to draw conclusions with regards to the general population of people with T2D. Apart from being treated with SGLT2i and GLP1RA, our patients received metformin and other drugs such as statins for treatment of their comorbidities. An additional impact of these other medications on meta-inflammation can therefore not be excluded. Finally, our study did not distinguish between people of different genders and race, which should be take into account when considering the translatability to the general population.

Conclusions

Our findings show that treatment of patients with type 2 diabetes with SGLT2 inhibitors alone or in combination with GLP1 receptor agonists ameliorates meta-inflammation mediated with by γδ T cells. This effect correlated with improved insulin sensitivity and a reduction in fat mass. Our findings may explain why these drugs have an anti-inflammatory, cardioprotective effect, despite being primarily used to lower blood glucose levels.

Declarations

Ethical approval and consent to participate

The ethics committee of the Institutional Review Board at Thalassotherapia Opatija approved this research under number 01-000-00-503/2-2020. We conducted the research in accordance and agreement with the International Conference on Harmonization Good Clinical Practice Guidelines and with the Declaration of Helsinki. All participants were over 18 years old and provided informed consent prior to inclusion in the study.

Consent for publication

All authors have read the manuscript, agree with its contents and consent with publication of its findings

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors 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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Authors' contributions

MTC: Investigation, Data Curation, Visualization, **DS**: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Visualization. **IK**: Investigation, Data Curation, Visualization, **VP:** Conceptualization, Resources, Writing - Review & Editing, **FMW**: Conceptualization, Methodology, Formal

analysis, Resources, Writing, Supervision, Project administration, Funding acquisition. **TTW**: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. All the authors revised the article critically for important intellectual content and finally approved the version to be published.

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Figures

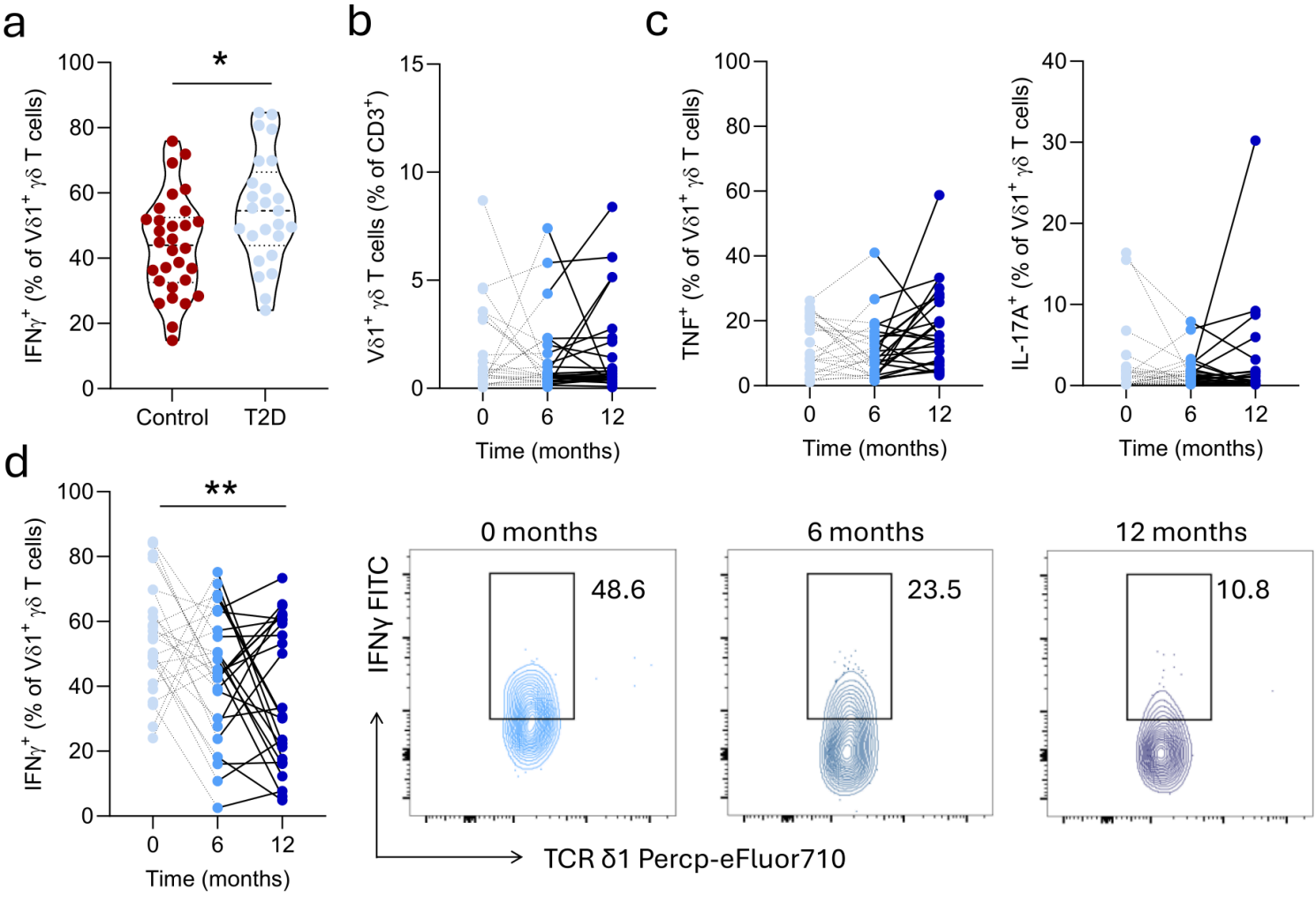


Figure 1

Antidiabetic treatment ameliorates IFNγ production by δ_1^+ γδ T cells. *(a) Peripheral blood mononuclear cells from patients with type 2 diabetes mellitus and matched controls were stimulated and interferon (IFN) γ production by δ_1^+ γδ T cells was determined using intracellular flow cytometry at baseline. (b) δ_1^+ γδ T cells as a frequency of total $CD3^+$ T cells after antidiabetic treatment. (c) Tumor necrosis factor (TNF) and interleukin (IL) 17A production by peripheral blood δ_1^+ γδ T cells upon in vitro restimulation after indicated times of antidiabetic treatment. (d) IFNγ production by peripheral blood δ_1^+ γδ T cells upon in vitro restimulation after indicated times of antidiabetic treatment. Shown are representative fluorescence activated cell sorting plots of $CD45^+CD3^+\delta_1^+$ cells from the same patient over time. An individual point represents one participant. Indicated are mean values ± standard error* of mean and statistical significances at *p<0.050 and **<0.010 by (a) Mann-Whitney and (d) one-way ANOVA with Tukey's multiple comparisons post testing.

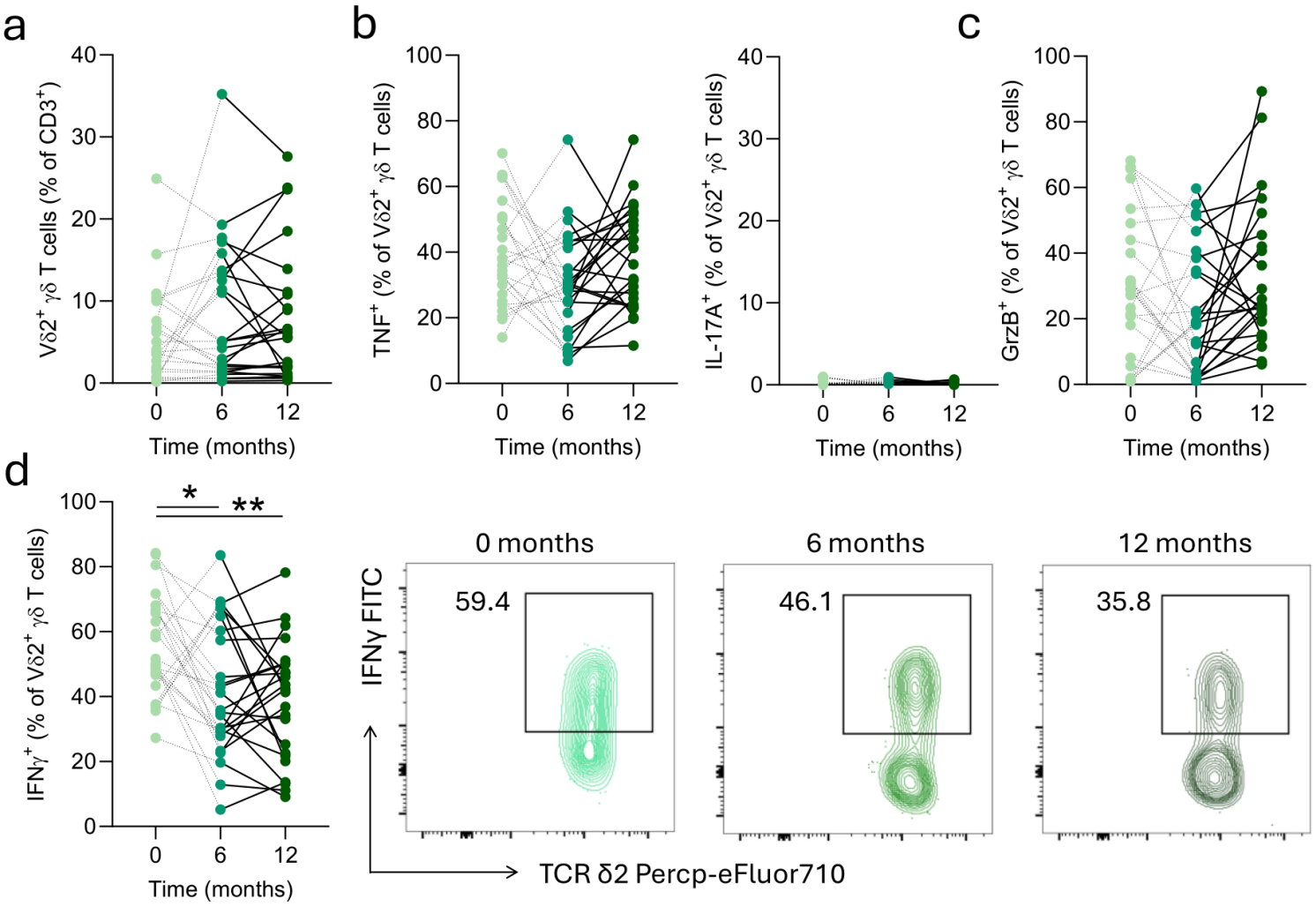


Figure 2

Antidiabetic treatment ameliorates IFNγ production by δ_2^+ γδ T cells. *(a) δ_2^+ γδ T cells as a frequency of total $CD3^+$ T cells after antidiabetic treatment. (b) Tumor necrosis factor (TNF) and interleukin (IL) 17A and (c) Granzyme B production by peripheral blood δ_2^+ γδ T cells upon in vitro restimulation after indicated times of antidiabetic treatment. (d) Interferon (IFN) γ production by peripheral blood δ_2^+ γδ T cells upon in vitro restimulation after indicated times of antidiabetic treatment. Shown are representative fluorescence activated cell sorting plots of $CD45^+CD3^+\delta_2^+$ cells from the same patient over time. An individual point represents one participant. Indicated are mean values ± standard error of mean and statistical significances at *p<0.050 and **<0.010 by one-way ANOVA with Tukey's multiple comparisons post testing.*

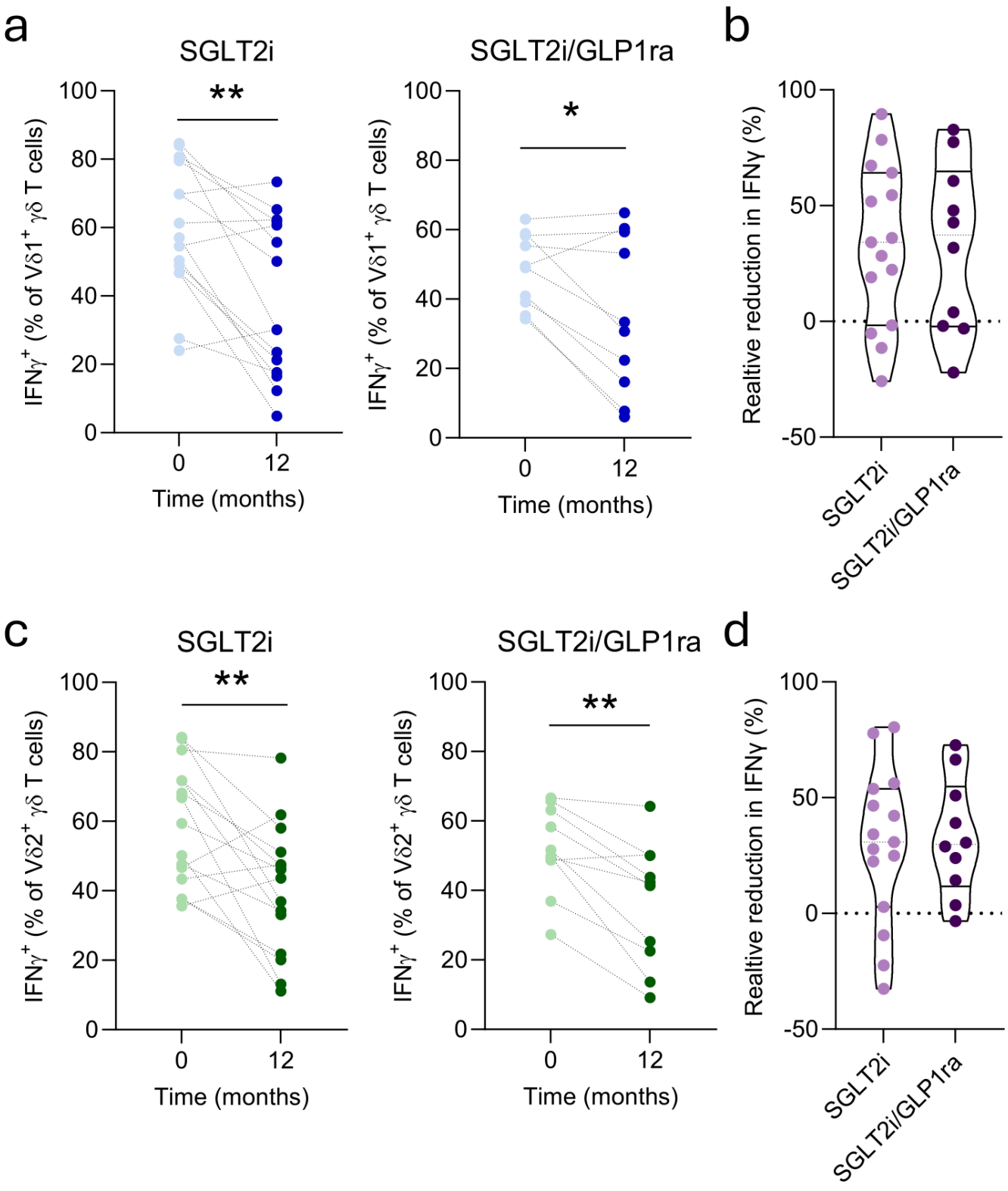


Figure 3

Combined treatment does not further ameliorate meta-inflammation. *Groups were segregated based on treatment regime and interferon (IFN) γ production was assessed using intracellular flow cytometry. (a) IFNγ production by δ_1^+ γδ T cells at 0 and 12 months. (b) Relative reduction in IFNγ production by δ_1^+ γδ T cells. (c) IFNγ production by δ_2^+ γδ T cells at 0 and 12 months. (d) Relative reduction in IFNγ*

*production by δ_2^+ γδ T cells. An individual point represents one participant. Indicated are mean values ± standard error of mean and statistical significances at *p<0.050 and **<0.010 by paired t test.*

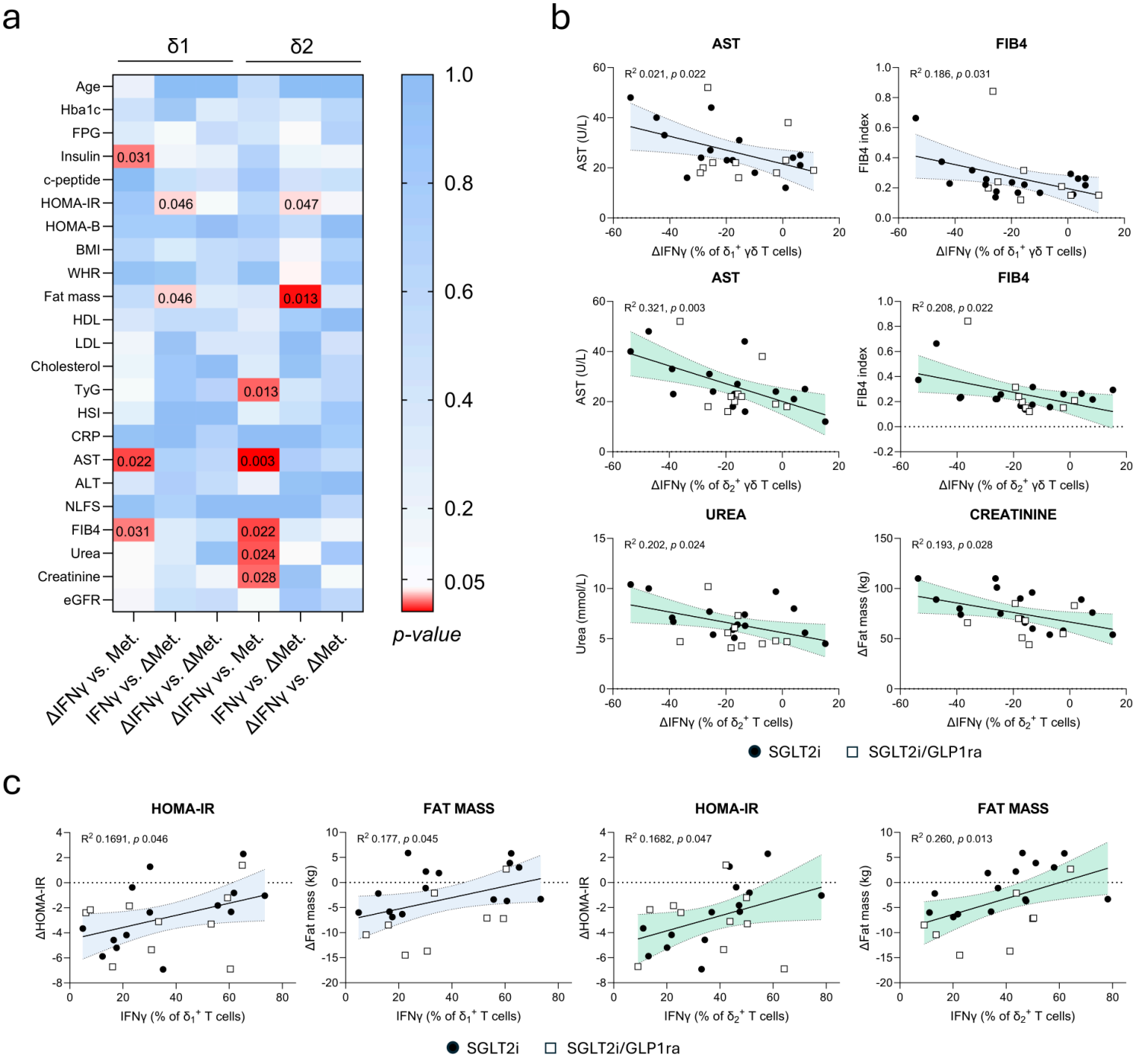


Figure 4

Reduced meta-inflammation correlates with reduced HOMA-IR and fat mass. Post hoc analysis of correlation between absolute levels of interferon (IFN) γ production and metabolic parameters, or relative change of these values (Δ) after 12 months of treatment. (a) Heat map of p values. Statistically significant changes are provided as numerical values. (b) Regression analysis correlating changes in IFNγ production with metabolic parameters. (c) Regression analysis correlating IFNγ production with changes in metabolic parameters. Regression analyses with statistically significant *p* value at <0.050

represented as mean and 95% confidence area of the best-fit line. ALT, alanine aminotransferase; AST, aspartate transaminase; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate FIB4, fibrosis-4 index; FPG, fasting plasma glucose; HbA_{1c}, glycated hemoglobin A_{1c}; HDL, high-density lipoprotein; HOMA-B, homeostatic model assessment beta cell function; HOMA-IR, homeostatic model assessment insulin resistance; HSI, hepatic steatosis index; LDL, low-density lipoprotein; NLFS, non-alcoholic fatty liver disease liver fat score; TyG triglyceride index; WHR, waist-hip ratio.

Supplementary Files

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