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**RESEARCH ARTICLE**

# Sodium–glucose cotransporter 2 inhibitors influence skeletal muscle pathology in patients with heart failure and reduced ejection fraction

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**Aims** Patients with heart failure and reduced ejection fraction (HFrEF) exhibit skeletal muscle pathology, which contributes to symptoms and decreased quality of life. Sodium–glucose cotransporter 2 inhibitors (SGLT2i) improve clinical outcomes in HFrEF but their mechanism of action remains poorly understood. We aimed, therefore, to determine whether SGLT2i influence skeletal muscle pathology in patients with HFrEF.

**Methods and results** Muscle biopsies from 28 male patients with HFrEF (New York Heart association class I–III) treated with SGLT2i (>12 months) or without SGLT2i were compared. Comprehensive analyses of muscle structure (immunohistochemistry), transcriptome (RNA sequencing), and metabolome (liquid chromatography-mass spectrometry) were performed, and serum inflammatory profiling (ELISA). Experiments in mice ($n = 16$) treated with SGLT2i were also performed. Myofiber atrophy was ~20% less in patients taking SGLT2i ($p = 0.07$). Transcriptomics and follow-up measures identified a unique signature in patients taking SGLT2i related to beneficial effects on atrophy, metabolism, and inflammation. Metabolomics identified influenced tryptophan metabolism in patients taking SGLT2i: kynurenic acid was 24% higher and kynurenine was 32% lower ($p < 0.001$). Serum profiling identified that SGLT2i treatment was associated with lower ($p < 0.05$) pro-inflammatory cytokines by 26–64% alongside downstream muscle interleukin (IL)-6-JAK/STAT3 signalling ($p = 008$ and 0.09). Serum IL-6 and muscle kynurenine were correlated ($R = 0.65$; $p < 0.05$). Muscle pathology was lower in mice treated with SGLT2i indicative of a conserved mammalian response to treatment.

**Conclusions** Treatment with SGLT2i influenced skeletal muscle pathology in patients with HFrEF and was associated with anti-atrophic, anti-inflammatory, and pro-metabolic effects. These changes may be regulated *via* IL-6–kynurenine signalling. Together, clinical improvements following SGLT2i treatment in patients with HFrEF may be partly explained by their positive effects on skeletal muscle pathology.

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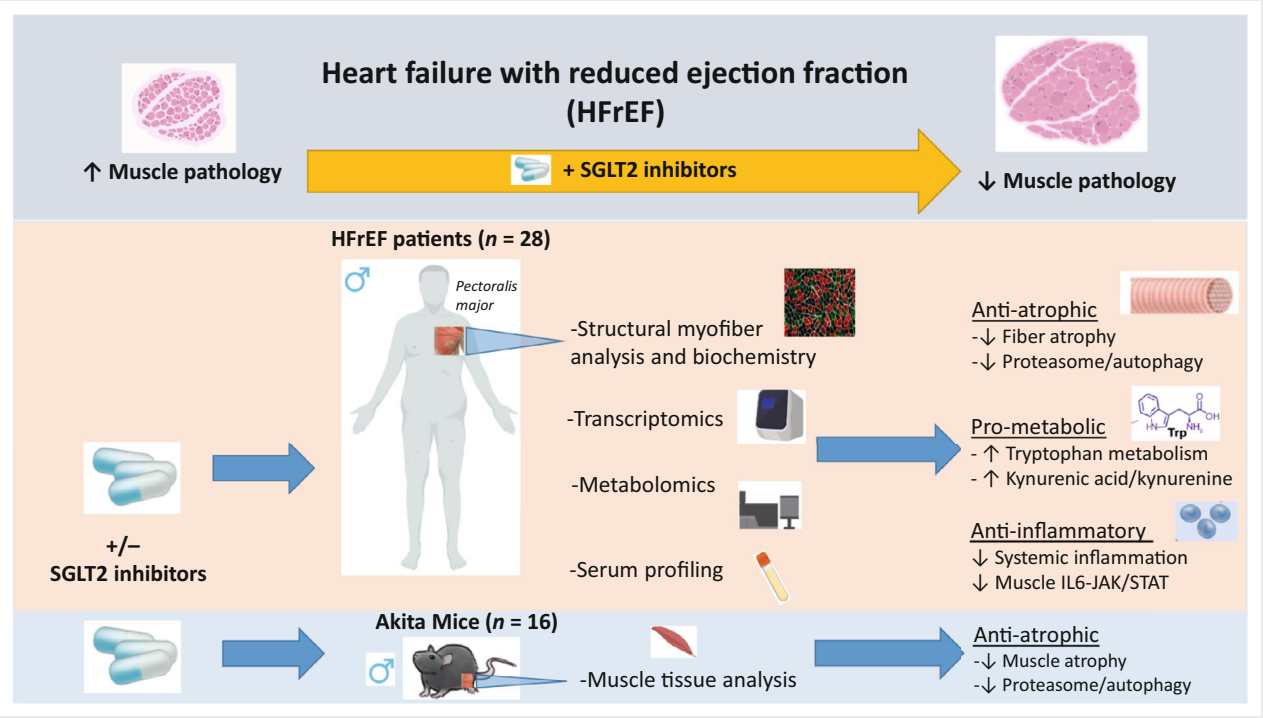
## Graphical Abstract




This study found that treatment with sodium–glucose cotransporter 2 (SGLT2) inhibitors in patients with heart failure and reduced ejection fractions (HFrEF) was associated with lower skeletal muscle pathology. Muscle biopsies were collected from patients treated with SGLT2 inhibitors (>12 months) and compared to untreated controls. State-of-the-art techniques were employed (from structural imaging to metabolomics), which revealed SGLT2 inhibitor treatment was associated with various improvements in muscle health. Additional experiments in mice treated with SGLT2 inhibitors confirmed positive effects of these drug agents on muscle health.

**Keywords** SGLT2 inhibitors • Muscle • Metabolism • Atrophy • HFrEF

# Introduction

Patients with heart failure and reduced ejection fraction (HFrEF) have a high symptom burden, low quality of life, and poor survival rates.[1] Exercise intolerance is the major symptom in HFrEF, but only part of this can be explained by cardiac (central) dysfunction.[2] Peripheral skeletal muscle pathology is considered a key therapeutic target in HFrEF,[2] as it directly exacerbates symptoms and independently predicts survival.[3] Muscle pathology in HFrEF is characterized by fiber atrophy and weakness alongside early fatigue, consequent to impaired energy metabolism, and abnormal fiber type shifts (Type I to II).[2] Underpinning this muscle pathology are a variety of mechanisms including elevated protein degradation (e.g. *via* MuRF1), pro-inflammatory cytokines (e.g. *via* interleukin-6 [IL-6], tumour necrosis factor (TNF)-α), reactive oxygen species, and mitochondrial dysfunction.[2] Treatments that slow, or even reverse, the progression of skeletal muscle pathology could offer an opportunity to improve clinical outcomes in HFrEF. However, to date, there remains no established pharmacological treatment for skeletal muscle pathology in HFrEF.

Sodium–glucose cotransporter 2 (SGLT2) inhibitors (SGLT2i) reduce the risk of cardiovascular death and hospitalizations for patients who have HFrEF with or without type 2 diabetes mellitus (DM).[4,5] SGLT2i also improve health-related quality of life, decrease symptoms, and increase physical performance (i.e. peak oxygen consumption) in heart failure.[6,7] However, the mechanisms underlying these effects remain poorly understood. Whereas SGLT2 proteins are primary regulators of the reabsorption of glucose back into the circulation from the glomerular filtrate by the kidneys, and their inhibition decreases blood glucose levels, they seem to have broader multi-organ effects *via* both direct and indirect mechanisms.[8] For example, in patients SGLT2i improve renal outcomes,[9] cardiac function and metabolism,[10] in addition to whole-body metabolism related to fatty acids (i.e. acylcarnitines, free fatty acids, ketone bodies) and amino acids (i.e. tryptophan).[8]

However, their possible effects on skeletal muscle pathology in patients with HFrEF remain unknown.

Based on previous work in rodent models showing that SGLT2i influence skeletal muscle homeostasis,[11–13] we hypothesized that skeletal muscle pathology would be less in patients with HFrEF taking SGLT2i due to anti-atrophic, pro-metabolic, and anti-inflammatory effects. We also performed independent mouse experiments to verify drug effects and hypothesized that SGLT2i muscle effects would be conserved across species.

# Methods

All experimental details are fully described in online supplementary material and summarized in the *Graphical Abstract*. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Participants

All participants provided written informed consent and all procedures were conducted in accordance with the Declaration of Helsinki after receiving ethical approval (11/YH/0291). Male patients treated with ($n = 12$) or without SGLT2i ($n = 16$) with established HFrEF (i.e. persistent symptoms and a left ventricular ejection fraction <40%) undergoing routine cardiac implantable electronic device (CIED) implantation at Leeds Teaching Hospitals were approached to take part in this study. Patients taking SGLT2i received either dapagliflozin or empagliflozin for a minimum of 12 months before muscle biopsy.

## Skeletal muscle biopsy

During CIED implantation, skeletal muscle biopsies from the *pectoralis major* were taken from each patient and frozen immediately in liquid nitrogen for subsequent analysis that included structural imaging, transcriptomics, metabolomics, gene and protein expression, and serum profiling.

## Structural imaging

Muscle samples were sectioned, stained and imaged to determine myofiber specific properties (size, type, capillaries).

## RNA sequencing and quantitative polymerase chain reaction

RNA was isolated from muscle samples and cDNA synthesized. Non-biased RNA sequencing (RNAseq) was performed using Illumina NovaSeq 6000 PE 150-bp and subjected to differentially expressed gene (DEG) analysis (DESeq2 v1.40), and gene ontology enrichment (ClusterProfiler v4.6.0). Quantitative polymerase chain reaction (qPCR) was also used to assess mRNA expression of target genes (online supplementary *Table S1*).

## Immunoblotting

Muscle samples were homogenized and expression of selected proteins was quantified via immunoblotting (online supplementary *Table S2*).

## Metabolomics

Targeted metabolomic analysis of acylcarnitines, tryptophan metabolites, free fatty acids and bile acids was conducted using liquid chromatography-mass spectrometry.

## Serum

Blood samples were collected and serum isolated for cytokine and chemokine analysis using the V-PLEX human cytokine 30-plex kit (Mesoscale).

## Mouse model

To compare effects across species, a similar experimental design was performed in a mouse model (Akita strain) fed normal chow ($n = 8$) or chow containing empagliflozin (0.045% $n = 8$) for 5 weeks. The gastrocnemius was harvested to assess changes in muscle atrophy and mRNA expression.

## Statistical analysis

Data were analysed in Prism (GraphPad Prism 9 v9.4.1). Maurice test of sphericity was conducted to assess normality and the respective parametric or non-parametric tests performed. Two-tailed *t*-tests were conducted to assess differences between groups unless stated otherwise and Fisher's exact tests were conducted for clinical variables. Spearman's or Pearson's correlation analysis was conducted to identify associations between variables. Statistical significance was accepted at $p < 0.05$ and data are presented as mean ± standard error of the mean unless stated otherwise.

# Results

## Patients

Patient characteristics are shown in *Table 1*. Those treated with SGLT2i were not different from those not receiving SGLT2i in terms of baseline characteristics.

## Structural muscle atrophy is lower following sodium–glucose cotransporter 2 inhibitor treatment

We first evaluated structural differences in skeletal muscle samples between patients with HFrEF treated with or without SGLT2i by examining cryosections carefully prepared from explanted biopsies. Representative images of myofibers from the different patient groups are shown in *Figure 1A*, with myofiber size tending to be higher ($p = 0.07$) in SGLT2i patients by 17% (*Figure 1B*). Further analyses identified predominant oxidative Type I fiber size was 23% higher in patients treated with versus without SGLT2i ($p = 0.07$), whereas similar trends were found in the more glycolytic Type IIA (20%) and Type IIX (12%) fibers albeit without attaining statistical significance ($p = 0.10$, $p = 0.15$; *Figure 1B*). Average myofiber atrophy did not increase as a function of age, either in each group (controls $p = 0.68$ and $r = -0.12$; or treated with SGLT2i $p = 0.75$ and $r = 0.14$) or across all patients ($p = 0.14$ and $r = -0.32$). This

**Table 1 Patient clinical characteristics**

| Patient characteristic | Controls ($n = 16$) | SGLT2i ($n = 12$) |
|---|---|---|
| Age (years) | 73 ± 2 | 66 ± 3 |
| Weight (kg) | 88 ± 4.3 | 88 ± 6.1 |
| Height (m) | 1.7 ± 0.015 | 1.7 ± 0.012 |
| BMI (kg/m$^2$) | 29 ± 1.4 | 29 ± 1.9 |
| NYHA class | | |
| I | – | 8.3% |
| II | 75% | 58.3% |
| III | 25% | 33.3% |
| LVEF (%) | 27 ± 2.3 | 22 ± 2.3 |
| NT-proBNP (ng/L) | 4052 ± 1243 | 2039 ± 799 |
| HbA1c (mmol/mol) | 48 ± 3.2 | 52 ± 6.6 |
| Comorbidities | | |
| Diabetes | 9 (56%) | 7 (58%) |
| COPD | 1 (6%) | 0 (0%) |
| HTN | 3 (19%) | 3 (25%) |
| AF | 7 (44%) | 3 (25%) |
| DCM | 2 (13%) | 4 (33%) |
| IHD | 9 (56%) | 7 (58%) |
| SGLT2i | | |
| Dapagliflozin | | 8 (67%) |
| Empagliflozin | | 3 (25%) |
| Canagliflozin | | 1 (8%) |

Data presented as mean ± standard error of the mean.
AF, atrial fibrillation; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DCM, dilated cardiomyopathy; HbA1c, glycated haemoglobin; HTN, hypertension; IHD, ischaemic heart disease; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; SGLT2i, sodium–glucose cotransporter 2 inhibitor.

lack of relationship between age and atrophy was present across all fiber subtypes. The trend for higher myofiber size after SGLT2i treatment was not associated with changes in fiber type (*Figure 1C*) or muscle capillarity (*Figure 1D*; online supplementary *Figure S1*). Together, these data suggest SGLT2i treatment was associated with lower muscle atrophy in patients with HFrEF without changes to muscle composition or capillarity.

## Sodium–glucose cotransporter 2 inhibitor treatment promotes an anti-atrophic muscle transcriptome signature

We found muscle atrophy was less in patients taking SGLT2i. We next explored underlying mechanisms by performing high-throughput non-bias RNAseq in collected muscle samples (*Figure 2A*). Our analysis identified 14 unique and significantly differentially expressed genes (DEGs) between patients taking SGLT2i, with eight DEGs up- and six DEGs down-regulated (adjusted $p < 0.05$; *Figure 2B*). Based on past evidence for a role in myofiber atrophy and inflammation, we identified and validated 5 of 14 genes *via* qPCR (PEX11A, AHRR, IGFBP2; SFRP5, GREM2; *Figure 2C,D*). Our analysis subsequently confirmed that IGFBP2, AHRR, and SFRP5 were all down-regulated with SGLT2i (*Figure 2C*) with each gene previously implicated in muscle pathology.

Our structural and transcriptomic data indicated SGLT2i impacts skeletal muscle atrophy in HFrEF. Next, we measured gene expression of the major atrophic pathways known to be up-regulated in HFrEF, the ubiquitin proteasome and autophagy systems.[2] Expression of ubiquitin proteasome genes were less in patients treated with SGLT2i (*Figure 3A*), with muscle-specific RING finger protein 1 (MuRF1) 60% lower ($p = 0.01$) and ubiquitin B (UBB) 66% lower ($p < 0.01$). In addition, expression of autophagy-dependent genes was lower with SGLT2i treatment (*Figure 3A*), with microtubule associated protein 1 light chain 3 beta (LC3B) 45% less ($p = 0.02$) and autophagy-related 7 (ATG7) 69% less ($p < 0.01$). Myofiber size is controlled by a tight coupling between catabolic and anabolic signalling, including a contributory role of muscle stem cells. Although expression of the pro-anabolic insulin-like growth factor 1 (IGF1) was not different between groups, nor the pro-catabolic factor myostatin (*Figure 3B*), expression of myogenic genes involved in muscle repair such as Notch1 and myogenin were lower ($p < 0.01$) with SGLT2i treatment (*Figure 3B*). These data indicate muscle damage–repair cycles may occur in HFrEF as in other diseases,[14] and that SGLT2i may decrease this. Together, our data indicate SGLT2i treatment is associated with lower atrophic, inflammatory, and injury signalling that could explain increased homeostasis at the myofiber structural level. Whether SGLT2i exert direct or indirect beneficial effects in HFrEF is controversial.[8] To confirm if sodium–glucose cotransporter (SGLT) receptors were expressed in skeletal muscle of our patients, we assessed gene expression of both SGLT1 (SLC5A1) and SGLT2 (SLC5A2) receptors. We detected lower expression of both SGLT1 and SGLT2 in skeletal muscle in patients taking SGLT2i (*Figure 3C*). These data indicate that SGLT2i may potentially have direct effects on skeletal muscle and the possible involvement of both SGLT receptors regulating muscle homeostasis.

To strengthen our observations in patients with HFrEF that SGLT2i treatment was associated with lower muscle atrophy and exclude potential confounding factors related to clinical differences (e.g. baseline variables, physical activity levels, treatment duration, and/or the muscle group sampled), we performed further experiments under highly controlled conditions in a cardiometabolic diabetic mouse model treated with or without SGLT2i for 6 weeks. We confirmed that skeletal muscle atrophy was attenuated in mice treated with SGLT2i compared to placebo (*Figure 3D*). Less atrophy was linked to decreases in both proteasome- and autophagy-dependent gene expression ($p < 0.02$; *Figure 3E*) but without changes in myogenic genes ($p > 0.05$; *Figure 3F*). Together, these data corroborate that SGLT2i beneficial effects on skeletal muscle pathology in disease represents a conserved mammalian response.

## Muscle tryptophan metabolism is improved after sodium–glucose cotransporter 2 inhibitor treatment

It has been proposed that SGLT2i regulate whole-body and cardiac metabolism.[15] Patients with HFrEF have impaired skeletal

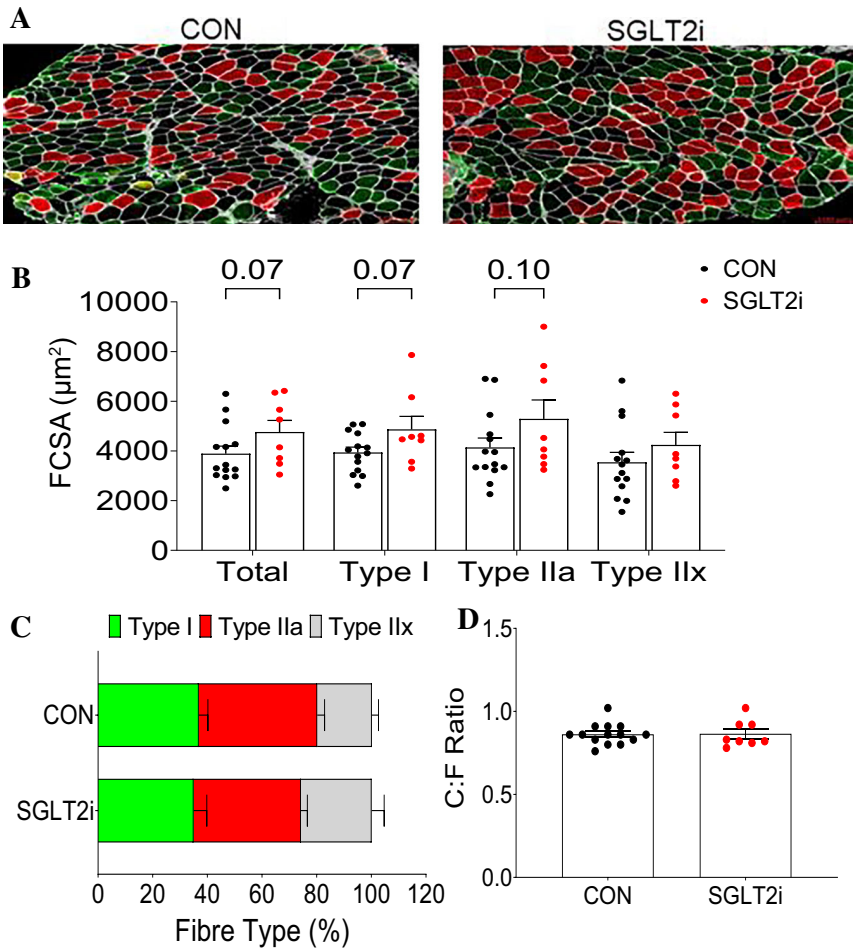
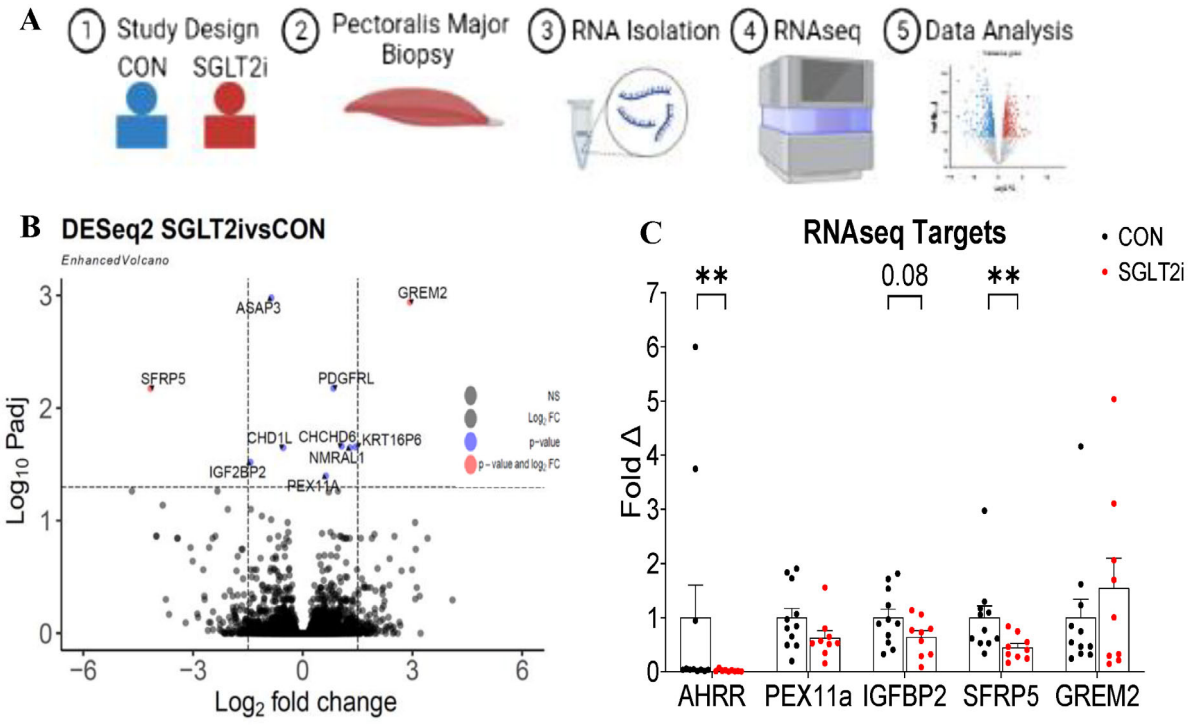



**Figure 1** (*A*) Representative stained cryosections of skeletal muscle biopsies from patients with heart failure and reduced ejection fraction that either received sodium–glucose cotransporter 2 inhibitors (SGLT2i) ($n = 14$) or did not (controls [CON]; $n = 8$), with myofiber morphology stained with antibodies as following: red = Type I fibers; green = Type IIa fibers; unstained fibers = Type IIx. (*B*) Quantified myofiber-specific size, termed fiber cross-sectional area (FCSA) with *p*-values presented. (*C*) Fiber type proportion (%). (*D*) Muscle capillarity (capillary-to-fiber [C:F] ratio). Dare are individual points and mean ± standard error of the mean.

muscle bioenergetics[16] and our RNAseq analysis also identified perturbed metabolic genes corrected *via* SGLT2i (e.g. AHRR, PEX11A; *Figure 2D*). Based on this, we next performed targeted metabolomics on patient muscle samples in order to identify any distinct metabolic signatures between those taking or not taking SGLT2i (*Figure 4A*). We first assessed metabolites involved in glucose and fatty acid oxidation, including tricarboxylic acid cycle intermediates, acylcarnitines, free fatty acids, diacylglycerol and triacylglycerols, however these were unchanged (online supplementary *Tables S3–S5*). This follows a recent study analysing plasma samples in HFrEF patients treated with dapagliflozin.[15] In contrast, recent reports suggest that amino acid metabolism is an important pathway influenced by SGLT2i.[17]

As our RNAseq analysis returned peroxisome proliferator-activated receptor-α pathway activation (online supplementary *Figure S2*), which is linked to kynurenine conversion (a metabolite of the essential amino acid tryptophan previously associated with muscle atrophy and inflammation), we next investigated the tryptophan pathway.[18] We found tryptophan ($p = 0.069$) as well as kynurenine ($p < 0.01$) were lower by 28.7% and 31.9%, whereas kynurenic acid was higher by 24.3% ($p < 0.01$), in patients taking SGLT2i (*Figure 4B–D*). Subsequently, there was an increase of 73.4% in kynurenic acid to kynurenine ratio ($p < 0.01$), suggesting a preferential conversion of neurotoxic kynurenine to neuroprotective kynurenic acid.[19] The kynurenine to tryptophan ratio, however, remained unchanged (*Figure 4E,F*). Melatonin and tryptamine levels were unchanged between groups, suggesting only the kynurenine pathway was altered by SGLT2i (*Figure 4G,H*). Overall, these data identify that HFrEF patients treated with SGLT2i show differences in skeletal muscle metabolism, specifically a preferential shunt in tryptophan metabolism from kynurenine towards kynurenic acid – a process well established to have broad physiological benefits that extend beyond the muscle.[20]




**D**

| Gene Symbol | Log2FoldChange | Padj |
|---|---|---|
| PLAAT4 | 1.15568121 | 2.94E-05 |
| MYL10 | −6.826065268 | 0.000185 |
| C4orf54 | 0.842241913 | 0.000585 |
| ASAP3 | −0.859629065 | 0.001052 |
| AHRR | −6.134670125 | 0.001149 |
| GREM2 | 2.928943173 | 0.001149 |
| PDGFRL | 0.840723483 | 0.006646 |
| SFRP5 | −4.161672022 | 0.006695 |
| CHCHD6 | 1.06292743 | 0.021791 |
| KRT16P6 | 1.440918657 | 0.022174 |
| CHD1L | −0.535481247 | 0.022438 |
| NMRAL1 | 1.290037332 | 0.022438 |
| IGFBP2 | −1.432009096 | 0.030254 |
| PEX11A | 0.63211462 | 0.040062 |

**Figure 2** (*A*) Schematic of workflow for muscle RNA sequencing (RNAseq) in patients with heart failure and reduced ejection fraction that either received sodium–glucose cotransporter 2 inhibitors (SGLT2i) ($n = 5$) or did not (controls [CON]; $n = 5$). (*B*) Volcano plot of 24 396 analysed genes from RNAseq revealed 14 significantly differentially expressed genes (DEGs) in patents who took SGLT2i, adjusted *p*-value <0.05. Red and blue dots denote significant genes that had a $\log_2$ fold change of >1.5 or <−1.5. (*C*) Validation of five selected DEGs from the RNAseq data via real-time quantitative polymerase chain reaction. (*D*) Table of all DEGS with $\log_2$ fold changes and adjusted *p*-values. Data are individual points and mean ± standard error of the mean. $^{**}p < 0.01$.

## Systemic inflammation is less after sodium–glucose cotransporter 2 inhibitor treatment

To probe for potential upstream mechanisms explaining how SGLT2i influence skeletal muscle pathology in HFrEF, we next examined circulating pro-inflammatory cytokines. Elevated serum cytokines contribute towards muscle pathology in HFrEF[2] and SGLT2i treatment is associated with a lowering of inflammatory mediators.[21] In our patients, SGLT2i treatment was associated with lower serum IL-6 levels by 64.1% ($p = 0.043$; *Figure 5A*) – a direct mechanism known to induce muscle atrophy.[22] Further analysis showed SGLT2i treatment was associated with lower levels of serum IL-16 (26.2%; $p = 0.031$), IL-17A (48%; $p = 0.034$), monocyte

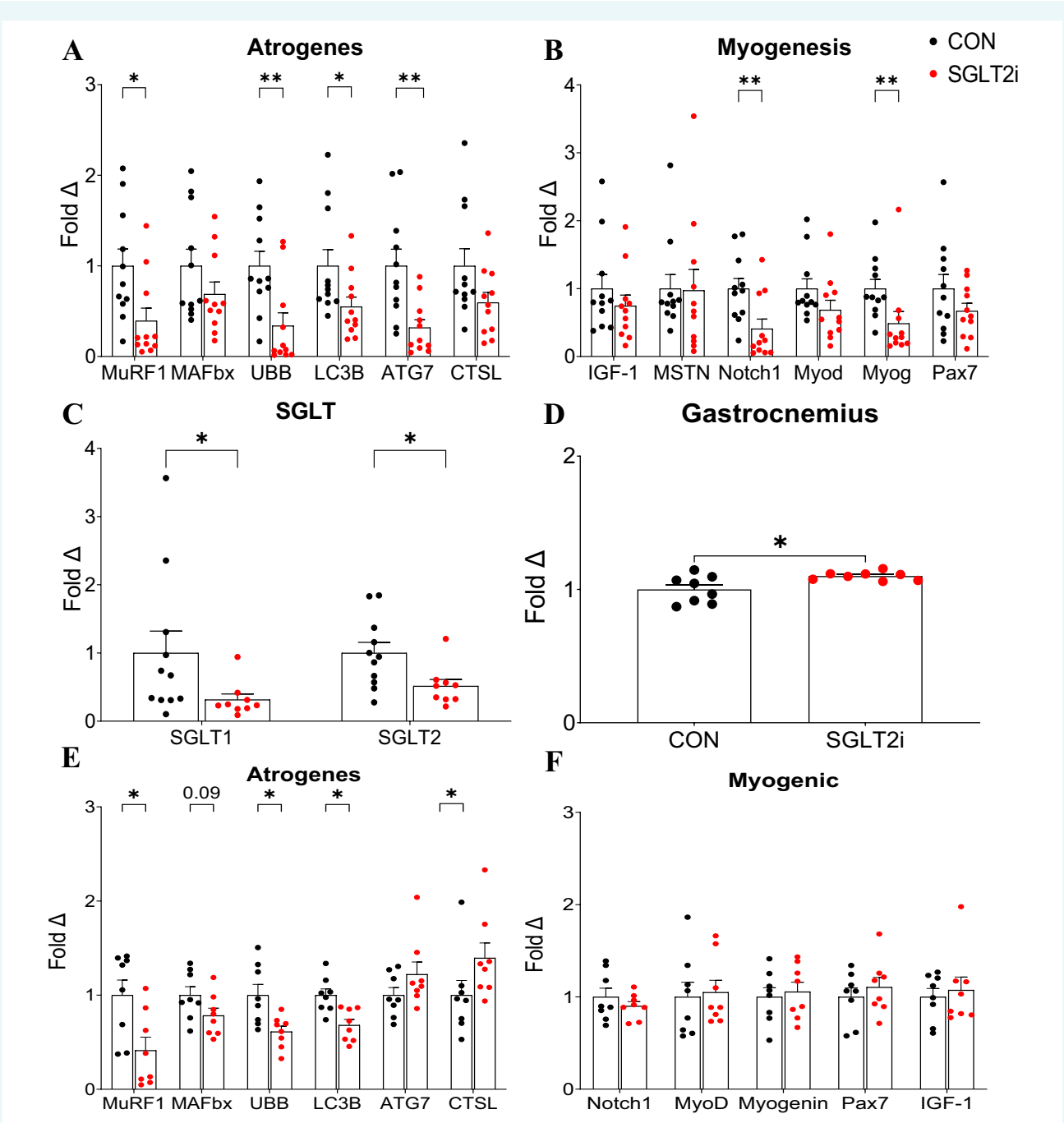



**Figure 3** Gene expression probed via quantitative polymerase chain reaction from muscle biopsies of patients with heart failure and reduced ejection fraction that either received sodium–glucose cotransporter 2 inhibitors (SGLT2i) ($n = 11$) or did not (controls [CON]; $n = 11$) for (*A*) atrogene, autophagy, inflammation, (*B*) myogenic markers, and (*C*) SGLT1 and 2 receptors. (*D*) Gastrocnemius skeletal muscle samples collected from mice with cardiometabolic disease treated with placebo (CON; $n = 8$) or SGLT2i ($n = 8$) were assessed for changes in (*D*) muscle mass; and gene expression for (*E*) atrophy signalling (*F*) myogenesis. Fold changes were normalised to the CON group. Data are individual points and mean ± standard error of the mean. *$p < 0.05$, **$p < 0.01$.

chemoattractant protein (MCP)-1 (48.8%, $p = 0.003$) and MCP-4 (41.9%; $p = 0.080$), whereas other pro-inflammatory cytokines were unaffected (IL-8, TNF, interferon-γ, angiotensin II; $p < 0.05$; *Figure 5A*).

Mechanistically, IL-6 can directly induce skeletal muscle atrophy via downstream JAK/STAT signalling.[2] To confirm if downstream atrophic pathways activated by IL-6 were lower after SGLT2i treatment, we used immunoblotting to investigate

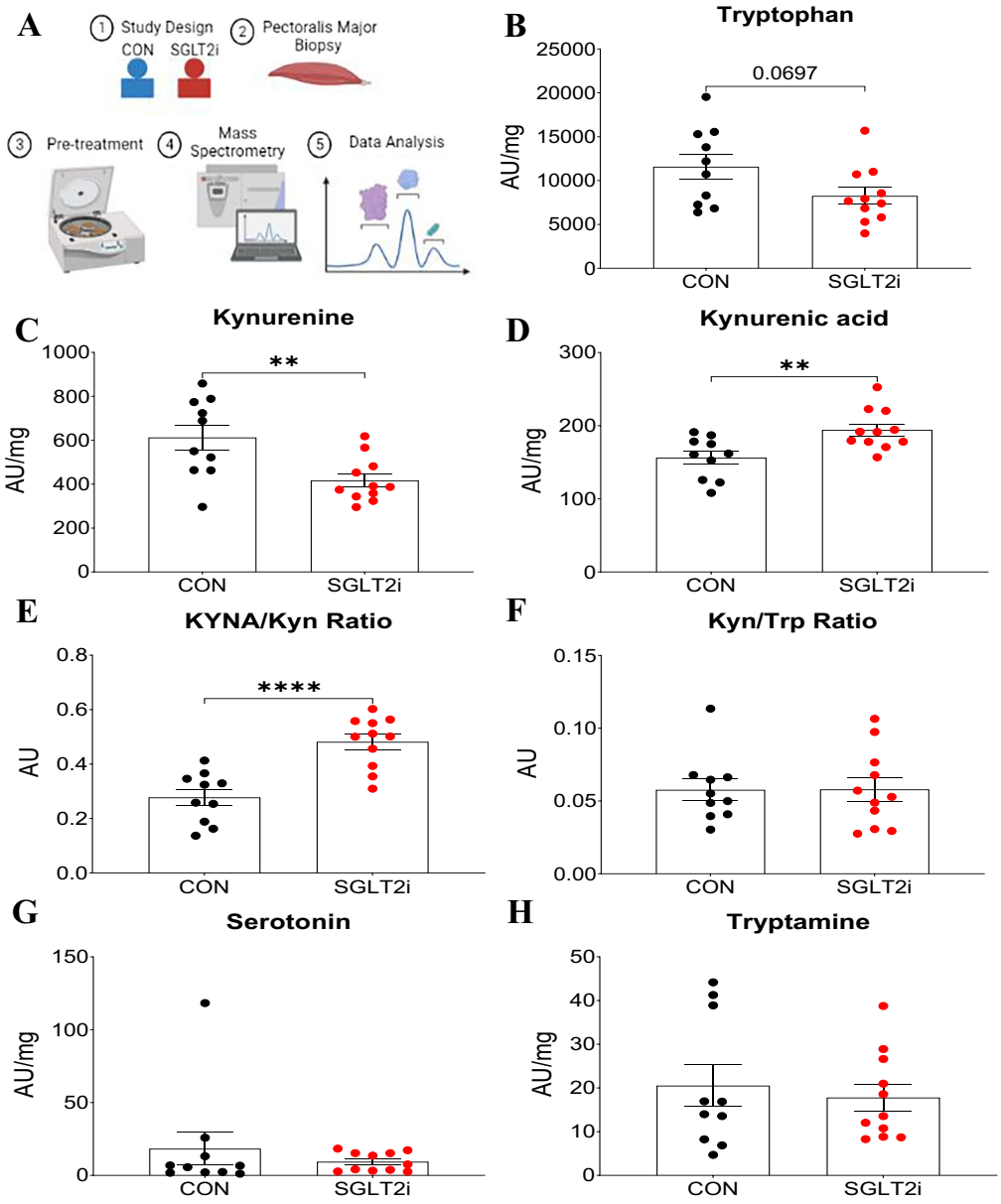



**Figure 4** (*A*) Schematic for liquid chromatography-mass spectrometry of muscle samples in patients with heart failure and reduced ejection fraction that either received sodium–glucose cotransporter 2 inhibitors (SGLT2i) ($n = 10$) or did not (controls [CON]; $n = 11$). Tryptophan metabolomics liquid chromatography-mass spectrometry data between groups. Data were normalized by integrating peaks to initial wet tissue mass in mg. Patients taking SGLT2i showed (*B*) lower tryptophan, (*C*) lower kynurenine, but (*D*) higher kynurenic acid, alongside with (*E*) higher ratio of kynurenic acid to kynurenine. (*F*) No changes were found between groups for (*F*) kynurenine to tryptophan ratio, (*G*) serotonin, or (*H*) tryptamine. Data are individual points and mean ± standard error of the mean. $^{**}p < 0.01$, $^{****}p < 0.001$.

protein phosphorylation status of STAT3, a key effector in the IL-6/JAK/STAT3 atrophic signalling pathway. HFrEF patients treated with SGLT2i tended to have lower expression of phosphorylated STAT3 by 20.1% and total STAT3 by 27.3% (both $p = 0.09$; *Figure 5B*). In line with this, we additionally confirmed muscle gene expression of IL-6 was lower after SGLT2i treatment by 55.6% ($p = 0.008$; *Figure 5C*). To provide further insight, correlation analysis between serum cytokines and myofiber markers was performed (online supplementary *Table S6*). Our approach found serum IL-6 and muscle kynurenine were strongly correlated (*Figure 5D*), amongst various others (online supplementary *Table S6*). Together, these data suggest that SGLT2i treatment in HFrEF patients is associated with lower systemic and local muscle-specific inflammation, and that inhibition of IL6-kynurenine signalling could be one mechanism by which SGLT2i attenuate muscle pathology.

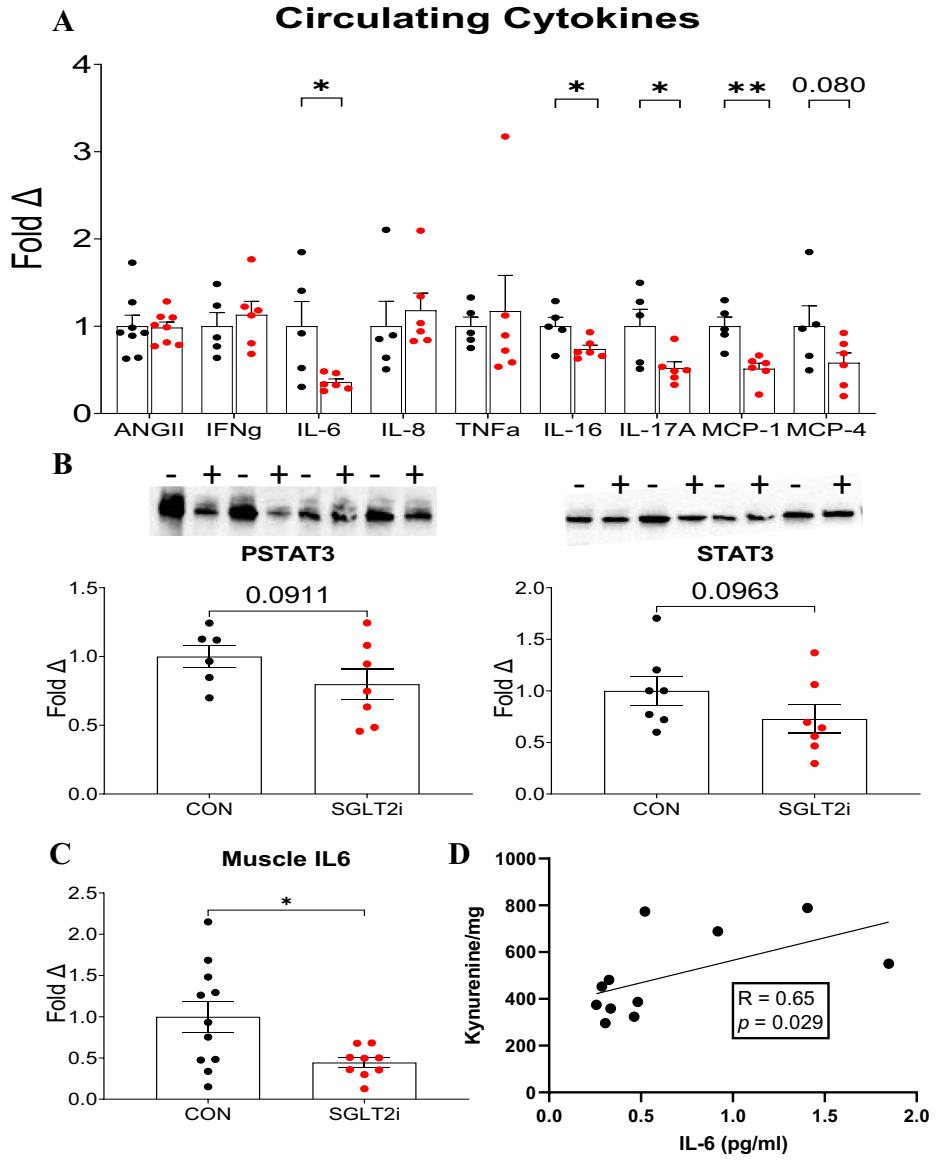



**Figure 5** (*A*) Serum analysis in patients ($n = 7–11$ per group) with heart failure and reduced ejection fraction that either received sodium–glucose cotransporter 2 inhibitors (SGLT2i) or did not (controls [CON]) for angiotensin II (ANGII), pro-inflammatory cytokines and chemokines. Interleukin (IL)-6, IL-16, IL-17A and monocyte chemoattractant protein (MCP)-1 were significantly lower in the SGLT2i group. (*B*) Western blot analysis in muscle samples of phosphorylated and total STAT3 normalized to Ponceau staining in CON ($n = 7$) and SGLT2i ($n = 7$) groups, with representative blots shown (− denoting CON and + denoting SGLT2i). (*C*) Gene expression of IL-6 in muscle samples from CON ($n = 11$) and SGLT2i ($n = 11$) groups. (*D*) Correlation between serum IL-6 and muscle kynurenine concentrations in patients. Data are individual points and mean ± standard error of the mean. IFNg, interferon-γ; TNFa, tumour necrosis factor-α. *$p < 0.05$, **$p < 0.01$.

## Discussion

This is the first study to investigate the effects of SGLT2i on skeletal muscle pathology in humans with heart failure. Skeletal muscle pathology is a key driver of symptoms, poor quality of life, and disease progression in HFrEF which, despite a great variety in symptomatic response to standard therapies and even in the face of improved heart function, often persists and for which there is no focussed treatment.[2] We comprehensively examined skeletal muscle biopsies collected from patients with stable HFrEF, treated with or without SGLT2i, and evaluated structural, transcriptome, metabolome, and inflammatory changes (*Graphical Abstract*). We provide evidence that muscle atrophy was lower in patients with HFrEF taking SGLT2i, which corresponded to lower proteasome- and autophagy-dependent signalling, shifts in tryptophan metabolism, and less inflammation. Mechanistically, our data highlight that SGLT2i may disrupt an IL-6-JAK/STAT-kynurenine signalling axis to attenuate muscle pathology in HFrEF patients.

Collectively, our proof-of-principle study suggests that one mechanism of action for SGLT2i could be via improving muscle pathology, and this may explain, at least in part, less symptoms and better quality of life in patients with HFrEF after SGLT2i treatment.[6,7]

## Anti-atrophic effects of sodium–glucose cotransporter 2 inhibitor in heart failure with reduced ejection fraction

In this study, SGLT2i treatment for >12 months was associated with less muscle atrophy by a clinically meaningful value of ~20%, as directly assessed in biopsies. These effects were independent of changes in fiber type or capillarity. No past studies have assessed the effects of SGLT2i on myofiber atrophy in patients with HFrEF. In HFrEF, two key pathways in skeletal muscle are upregulated to increase atrophy: the ubiquitin proteasome and the autophagy systems.[2] We observed lower expression in key genes involved in both the proteasome (MuRF1, UBB) and autophagy (LC3B, ATG7) systems, similar to changes seen after prolonged exercise training.[23] This may have important clinical relevance, as many patients are often too sick or frail to perform sustained exercise regimes, meaning alternative, well-tolerated pharmacological approaches are required. Given that SGLT2i provided a similar protection to muscle loss in our independent mouse experiments strengthens an anti-atrophic benefit of these drugs.

## Mechanisms of sodium–glucose cotransporter 2 inhibitor benefits on skeletal muscle in heart failure with reduced ejection fraction

To explore underlying mechanisms for why taking SGLT2i in HFrEF patients was associated with less muscle atrophy, we applied high throughput unbiased transcriptomics, targeted metabolomics, and inflammatory profiling. We identified a potential mechanism involving an IL-6/kynurenine axis that may offer a novel explanation for how SGLT2i mediate benefits on the skeletal muscle in HFrEF. Circulating IL-6 is an independent predictor of mortality in HFrEF patients, and serum concentrations are directly associated with adverse events[24] and greater muscle abnormalities.[2] In the current study, patients taking SGLT2i had lower circulating and skeletal muscle IL-6 levels. Genetic overexpression of IL-6 in mouse models directly causes skeletal muscle atrophy *via* IL-6-JAK/STAT pathway and this mediates atrogene-dependent protein degradation *via* MuRF1 and MAFBx, but also via autophagy.[25] Together, it is likely SGLT2i protect skeletal muscle in HFrEF by attenuating proteasome and autophagy-dependent atrophy via down-regulation of systemic cytokines such as IL-6 and local IL-6/STAT3 signalling.

Recently, IL-6 was shown to induce kynurenine synthesis *via* indoleamine 2,3-dioxygenase 1.[26] Kynurenine treated mice show increased muscle atrophy,[27] with IL-6–kynurenine axis linked to increased muscle atrophy in sepsis.[28] Plasma kynurenine is inversely associated with muscle strength and peak oxygen consumption in patients with HFrEF.[29] In the current study, patients with HFrEF taking SGLT2i demonstrated a shunt in muscle tryptophan metabolism, in the direction of lower kynurenine and higher kynurenic acid concentrations. Kynurenic acid reduces IL-6 and nuclear factor-κB expression in skeletal muscle, both of which are upstream inducers of atrogene MuRF1.[2] In this study, SGLT2i treated patients showed both lower kynurenine, and higher kynurenic acid (absolute and relative to kynurenine), which were strongly correlated to IL-6 serum expression. Taken together, our data show some similarities to changes induced via endurance exercise, which induces a shift of muscle tryptophan metabolism to kynurenic acid in healthy humans.[30] Therefore, we hypothesise that SGLT2i may attenuate muscle atrophy by reducing systemic IL-6 expression, leading to lower local skeletal muscle IL-6 expression but increased kynurenine to kynurenic acid conversion that ultimately decreases inflammation and atrophy. It remains unknown whether the SGLT2i signal to muscle is *via* direct or indirect effects, although we were able to detect the SGLT2 receptor in the muscle samples of HFrEF patients. Determining the exact signalling pathway requires future work.

## Limitations

Our findings may not be applicable to the general HFrEF population, as it was limited to a single centre, male patients, and a low sample size. However, accessing muscle biopsies represents an approach which is technically and ethically challenging. Most patients are not willing to undergo the perceived risks associated with muscle biopsy, which restricts sample size. In addition, given the approvals of the SLGT2i agents, we were unable to undertake a randomized controlled study, such that this was a retrospective analysis of previously collected samples. However, in terms of comparisons, the patients in each group were well matched for clinical and physical characteristics, and we further validated some of our findings in a mouse model. Patients taking SGLT2i were numerically (but not statistically) younger than untreated patients. However, as muscle atrophy did not increase as a function of age in our cohort, and as past studies have shown similar changes in muscle pathology between old and young patients with HFrEF,[23] age alone is unlikely to be a confounding factor. Finally, multiple SGLT2i treatments were included in this study, but this is only a limitation if the effects of one are greater or distinct than the other, however there is currently no evidence to support this.

# Conclusion

This is the first study to investigate the effects of SGLT2i on the skeletal muscle pathology in patients with HFrEF. Our data suggest that SGLT2i treatment is associated with anti-atrophic, anti-inflammatory, and pro-metabolic effects in skeletal muscle in HFrEF, all of which could contribute to the known clinical benefits of these agents. Further work is necessary to explore specific pathways and their contribution to the improved clinical outcomes.

# Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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