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Research article

# Metformin as a strategy against false positives in $^{18}$F-FDG PET/CT due to inflammation

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A B S T R A C T

Lung cancer is the leading cause of cancer-related deaths globally. Despite recent improvements in incidence and mortality rates, the prognosis of lung cancer remains dire.$^{18}$F-FDG PET/CT plays a vital role in diagnosing, staging, and monitoring the therapeutic efficacy of lung cancer. However, the high glucose metabolism in inflammatory lesions, driven by macrophage activation, aggregation, and the release of inflammatory factors, is a primary source of false-positive results in FDG PET/CT oncology. This significantly diminishes the specificity and accuracy of PET/CT in diagnosing and staging lung cancer.Here we show FoxO1 plays a role in glucose metabolism in macrophages.We found that metformin regulated FoxO1 expression in macrophages, regulated the expression of inflammatory mediators and apoptosis of macrophages, thus reducing inflammatory glucose metabolism and improving the diagnostic accuracy of $^{18}$F-FDG PET/CT in lung cancer.We anticipate that our study could provide a credible approach to improve tumor diagnostic accuracy with PET/CT.

## 1. Introduction

Globally, lung cancer is increasingly prevalent and remains the most common malignant tumor and the leading cause of cancer-related deaths. Recent advancements in understanding lung cancer and the selection of appropriate treatment modalities have improved its incidence and mortality rates. However, the prognosis is still grim, with a 5-year survival rate of only 20.5 % [1]. Therefore, early diagnosis and accurate staging are critical for effective lung cancer treatment.

$^{18}$F-FDG PET/CT is indispensable in diagnosing, staging, and monitoring the therapeutic efficacy of lung cancer, playing a pivotal role in managing lung cancer patients [2,3]. However, inflammation or infection-induced recruitment of inflammatory cells and their released mediators can also lead to FDG accumulation [4–8], resulting in false-positive results in $^{18}$F-FDG PET/CT [9-11]· This significantly undermines the specificity and accuracy of PET/CT in lung cancer diagnosis and staging. The local high glucose metabolism, driven by macrophage activation and aggregation in inflammatory lesions, is the primary cause of increased $^{18}$F-FDG uptake [7,8,12]. In the early stages of inflammation, activated macrophages exhibit high glucose metabolism. Upon entering the inflammatory site, they release chemokines and pro-inflammatory factors, further amplifying the inflammatory response and inducing more immune cells, including macrophages, to activate and aggregate [13].

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Metformin, a first-line drug for treating type 2 diabetes, is known for its high safety and low cost. Recently, its anti-inflammatory effects have been explored, showing efficacy through mitochondrial pathways and in treating inflammatory diseases such as rheumatism and enteritis [14–16]. We have summarized various anti-inflammatory mechanisms of metformin, including NF-κB, NLRP3 inflammasome, ATF-3, and others [14], and identified the FoxO family as playing a significant role in inflammatory and immune cells. We hypothesize that metformin, by regulating FoxO1, could modulate macrophage activity and the expression of inflammatory mediators, thereby reducing glucoglycolysis at inflammation sites. Validating this hypothesis could not only enhance the accuracy of PET/CT in diagnosing lung cancer but also provide new strategies for treating lung inflammation.

## 2. Materials and methods

### 2.1. Cell lines and culture conditions

The macrophage cell line U937 and the lung cancer cell line LLC were acquired from Meisen Chinese Tissue Culture Collections. U937 cells were cultured in RPMI-1640 medium (Gibco, USA), supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin solution. In contrast, LLC cells were maintained in DMEM medium (Gibco, USA) with the same supplements. Both cell lines were incubated in a humidified atmosphere containing 5 % $CO_2$ at a constant temperature of 37 °C.

### 2.2. Quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR)

Gene expression was analyzed following treatment with metformin (Macklin, China) and the FoxO1 inhibitor AS1842856 (Meilun Bio, China) using two-step reverse transcription-polymerase chain reaction quantitative assays. Total RNA was extracted from the cells using Beyozol reagent, followed by conversion to first-strand cDNA using a cDNA synthesis kit. The cDNA was then subjected to quantitative real-time PCR using the CFX96 fluorescence quantitative PCR instrument (Bio-Rad, USA), adhering to the manufacturer's instructions. Target gene levels were quantified relative to β-actin, serving as the reference gene. Primers for the target genes are detailed in Table 1. The thermal cycling conditions were as follows: an initial denaturation at 95 °C for 3 min, followed by 42 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s, and extension at 60 °C for 30 s. Messenger RNA (mRNA) levels were normalized to β-actin mRNA as an internal control. The primers for the target genes are listed in Table 1.

### 2.3. Western blotting analysis

Following treatment with metformin and the FoxO1 inhibitor AS1842856, cells were harvested and lysed using RIPA buffer containing 10 % PMSF. Proteins were then extracted, and their concentrations determined using the BCA protein assay kit (Beyotime, Shanghai, China).Samples were prepared with 5-protein loading buffer (Beyotime, Shanghai, China) and boiled in a metal bath for 10 min.Proteins were separated using 10 % and 15 % SDS-PAGE electrophoresis and subsequently transferred onto PVDF membranes. The membranes were blocked with 5 % nonfat milk powder for 1 h, followed by overnight incubation at 4 °C with primary antibodies. After washing thrice with TBST, the membranes were incubated with sheep anti-rabbit IgG HRP (Proteintech, USA) at room temperature for 2 h. Following three additional washes with TBST, the membranes were developed using a supersensitive ECL chemiluminescent solution (Beyotime, Shanghai, China).

### 2.4. Cell counting Kit-8 (CCK-8)

Cells were cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10 % fetal bovine serum. Post-treatment with metformin and the FoxO1 inhibitor AS1842856 for 48 h, cells were incubated with CCK-8 reagent (Glpbio, USA)in the incubator for 1 h. Absorbance at 450 nm was measured using an ELISA reader (Thermo Scientific, USA). Cell survival rate was calculated as follows: Cell survival rate (%) = [(As-Ab)/(Ac-Ab)] × 100 %, where As is the absorbance of the experimental well, Ab is the absorbance of the blank

**Table 1**
The primers used in this study.

| Gene | | Sequence(5′-3′) |
|---|---|---|
| β-actin | Reverse | CTCCTTAATGTCACGCACGAT |
| | Forward | CATGTACGTTGCTATCCAGGC |
| FoxO1 | Reverse | CGGCTTCGGCTCTTAGCAAA |
| | Forward | TCGTCATAATCTGTCCCTACACA |
| IL-1β | Reverse | CCTGGAAGGAGCACTTCATCT |
| | Forward | AGCCATGGCAGAAGTACCTG |
| IL-6 | Reverse | CCATCTTTGGAAGGTTCAGGTTG |
| | Forward | ACTCACCTCTTCAGAACGAATTG |
| TNF-α | Reverse | TGTGTATCGGTGCATGGTTTTA |
| | Forward | TCCTCAGGCTTTGTATTTGAGC |
| TGF-β1 | Reverse | TGACACAGAGATCCGCAGTC |
| | Forward | CTGTCCAACATGATCGTGCG |

well, and Ac is the absorbance of the control well.

### 2.5. Flow cytometry (FCM)

Cells were cultured in RPMI-1640 medium (Gibco, USA) containing 10 % fetal bovine serum at a density of 2-3 $\times$ 10^5/ml. After treatment with metformin and the FoxO1 inhibitor AS1842856, cells were collected and resuspended in PBS containing 0.1 % bovine serum albumin (BioFrocxx, Germany). Apoptosis was analyzed using the Annexin V-FITC/PI Apoptosis Kit (Elabscience, Wuhan, China) and flow cytometry (CytoFLEX, USA). Data were processed and analyzed using CytExpert 2.5.077.

### 2.6. In vitro $^{18}$F-FDG uptake assay

$^{18}$F-FDG was sourced from the Nuclear Medicine Department of the First Affiliated Hospital of Chongqing Medical University. U937 cells were cultured in glucose-free RPMI-1640 medium (Gibco, USA) containing 10 % fetal bovine serum. Post-treatment with metformin and the FoxO1 inhibitor AS1842856, cells were washed and resuspended in PBS. Each well received 1 ml of $^{18}$F-FDG (radioactive concentration: 3.7 $\times$ 10^6Bq/ml) and was incubated at room temperature for 30 min. Following centrifugation and washing, cell-associated CPM counts were measured using a γ-counter (PerkinElmer, USA). The impact of metformin and the FoxO1 inhibitor on macrophage $^{18}$F-FDG uptake was assessed and compared. Lung cancer cells were similarly treated to examine the effect of metformin on $^{18}$F-FDG uptake.

### 2.7. In vivo $^{18}$F-FDG PET/CT tumor imaging

All animal procedures were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University (IACUA-CQMU) (approval number: IACUC-CQMU-2023-0131). Wild C57 mice (aged 4–6 weeks; weighing 18–20g, Ensiweier, China) and FoxO1 gene knock-out C57 mice (Cyagen, China) were used. The mice were acclimatized under standard laboratory conditions (22 $\pm$ 2 °C, 50 % relative humidity) for one week. Wild mice were randomly divided into two groups (lung cancer, inflammation,n $\geq$ 3) with no significant differences in initial conditions. The FoxO1-KD mice were the FoxO1 gene konck-out group.A 10^6/ml suspension of LLC lung cancer cells in physiological saline was subcutaneously injected into the left flank of each mouse to establish a subcutaneous tumor model. Tumor growth was monitored, and once the volume reached 0.5–1.0 cm^3, 50 μl of turpentine oil (Macklin, China) was injected into the right thigh muscle to induce inflammation. Prior to imaging, mice were fasted overnight and housed individually. Each tumor-bearing mouse received an intraperitoneal injection of 100–200 μCi/100 μL of $^{18}$F-FDG. PET/CT data were collected 60 min post-injection using a micro PET/CT system (nanoScan PET/CT, USA). Metformin was administered intraperitoneally at 250 mg/kg for 2 days, while some mice in the inflammatory model group received an equivalent volume of saline. The procedure was repeated post-intervention. PET/CT images were analyzed using Bee Dicom Viewer (Sano United Medical Technology Co., Ltd, Beijing, China). Tumor and inflammation tracer uptake was quantified as the standardized uptake value (SUV), calculated as follows: SUV = tissue activity concentration (Bq/mL)/injected dose (Bq) $\times$ body weight (g).SUVmax was used for semiquantitative analysis, and the effect of metformin intervention on SUVmax before and after intervention was calculated and compared.

### 2.8. Tissue immunohistochemical staining (IHC)

Post-imaging, mice were euthanized with an intraperitoneal injection of 150 mg/kg sodium pentobarbital. Inflammation and tumor tissues were fixed in 4 % paraformaldehyde and embedded in paraffin. FoxO1 gene expression was assessed using immunohistochemical staining (Servicebio, China; Wuhan Servicebio Technology Co., Ltd, China). Slides were analyzed using CaseViewer, with DAB staining indicating positive staining (scale: 20 μm).

### 2.9. Construction of the FoxO1 knockout mouse model

The FoxO1 gene (NCBI Reference Sequence: NM_019739.3; Ensembl: NSMUSG00000044167) is located on mouse chromosome 3, comprising three exons with the ATG start codon in exon 1 and the TAA stop codon in exon 2 (Transcript Foxo1-201: ENSMUST00000053764). Exon 2 was targeted for knockout. Cas9 and gRNA were co-injected into fertilized eggs to produce KO mice. Offspring were genotyped by PCR and sequencing analysis. For FoxO1-KO heterozygous mice (Cyagen, China), FoxO1 gene expression levels were determined post-micro PET/CT imaging using Western blotting and RT-PCR.

### 2.10. Statistical analysis

Statistical analyses were conducted using SPSS 23 and GraphPad Prism 9. Results are presented as means $\pm$ SDs unless otherwise stated. One-way ANOVA or paired t-tests were used for normally distributed data, with the least significant difference (LSD) post hoc test for multiple comparisons.Nonparametric tests were used for non-normally distributed data. All statistical tests were two-tailed, and a P-value $<$0.05 was considered statistically significant.

## 3. Results

### 3.1. Effect of metformin on $^{18}$F-FDG uptake in macrophages

To explore the influence of metformin on $^{18}$F-FDG uptake in macrophages, we assessed the impact of varying intervention durations and concentrations of metformin. Our findings revealed that metformin effectively reduced $^{18}$F-FDG uptake in macrophages (Fig. 1). Notably, as the concentration of metformin incrementally increased from 0 to 1 mM, 5 mM, 10 mM, and finally to 20 mM, its efficacy in diminishing $^{18}$F-FDG uptake in macrophages progressively intensified, yielding P values of 0.0025, 0.0017, 0.0007, and 0.0002, respectively. Similarly, extending the intervention duration of metformin from 0 to 12 h, 24 h, and up to 48 h, progressively enhanced its effect in reducing $^{18}$F-FDG uptake in macrophages, with corresponding P values of >0.9999, 0.1823, 0.0021, and <0.0001. In subsequent experiments, lung cancer cells were treated with 10 μM metformin for 48 h. This treatment showed no significant impact on $^{18}$F-FDG uptake in lung cancer cells ($P = 0.6857$).

### 3.2. Metformin's mechanism in reducing $^{18}$F-FDG uptake in macrophages

In our previous review, we discussed the anti-inflammatory effects of metformin, emphasizing the pivotal role of FoxO1 in the inflammatory response [14]. To delve deeper into the specific mechanism by which metformin reduces $^{18}$F-FDG uptake in macrophages, we employed metformin and a FoxO1 inhibitor as interventions. We then measured the levels of macrophage apoptosis, FoxO1 expression, and the expression of downstream inflammatory mediators such as IL-1β, IL-6, TNF-α, and TGF-β1. As depicted in Fig. 2, both metformin and the FoxO1 inhibitor significantly enhanced macrophage apoptosis ($P < 0.0001$), with metformin increasing the apoptosis rate from 19.42 % to 41.46 %, and the FoxO1 inhibitor from 19.42 % to 31.81 %. Concurrently, both interventions markedly reduced the expression of FoxO1 in macrophages ($1 \pm 0$ vs $0.2301 \pm 0.03713$, $P < 0.0001$; $1 \pm 0$ vs $0.32101 \pm 0.1024$, $P < 0.0001$) and the pro-inflammatory cytokines IL-1β ($1 \pm 0$ vs $0.04483 \pm 0.0074$, $P < 0.0001$; $1 \pm 0$ vs $0.09404 \pm 0.02099$, $P < 0.0001$), IL-6 ($1 \pm 0$ vs $0.2687 \pm 0.1472$, $P < 0.0001$; $1 \pm 0$ vs $0.5127 \pm 0.1160$, $P < 0.0001$), and TNF-α ($1 \pm 0$ vs $0.24 \pm 0.05012$, $P < 0.0001$; $1 \pm 0$ vs $0.2678 \pm 0.08710$, $P < 0.0001$). Additionally, they promoted the expression of the anti-inflammatory cytokine TGF-β1 ($1 \pm 0$ vs $3.947 \pm 0.8415$, $P < 0.0001$; $1 \pm 0$ vs $3.435 \pm 1.070$, $P = 0.0009$).

### 3.3. Effects of metformin on $^{18}$F-FDG uptake in lung cancer and inflammation

To investigate the impact of metformin on $^{18}$F-FDG imaging in inflammatory conditions in mice, we conducted a series of experiments. As illustrated in Fig. 3, metformin intervention notably reduced $^{18}$F-FDG uptake at the inflammation sites in our mouse model ($3.940 \pm 0.374$ vs $2.645 \pm 0.9284$, $P = 0.0434$). However, no significant change was observed in FDG uptake in the inflamed lesions of mice treated with saline ($2.627 \pm 0.5582$ vs $3.083 \pm 0.2797$, $P = 0.1081$). Similarly, in the lung cancer tumor mouse model, metformin intervention did not significantly alter $^{18}$F-FDG uptake ($6.443 \pm 1.302$ vs $6.930 \pm 1.298$, $P = 0.6815$). These findings indicate that metformin can selectively reduce FDG uptake in inflammatory sites without impacting the uptake in lung cancer. In terms of molecular mechanisms, the expression of FoxO1 in the metformin-treated group was markedly decreased compared to the saline group, showing a 10-fold reduction ($0.5950 \pm 0.0695$ vs $0.0600 \pm 0.0100$).

### 3.4. Impact of FoxO1 knockdown on inflammation and $^{18}$F-FDG uptake

To further elucidate the role of FoxO1 in the anti-inflammatory effects of metformin and its implications in FDG PET/CT imaging, we developed FoxO1 knockout mice (The strategy for constructing FoxO1-KD mice is depicted in Fig. 4D). As demonstrated in Fig. 4,

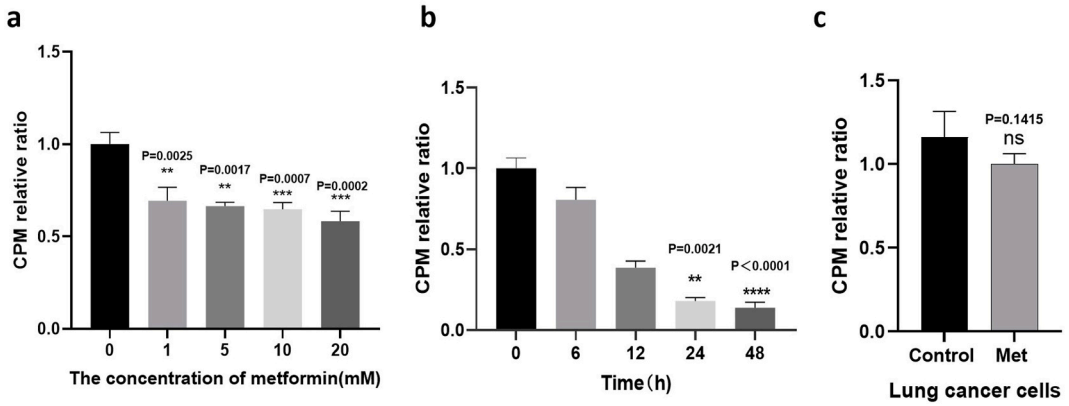


**Fig. 1.** A. Effect of different concentrations of metformin on $^{18}$F-FDG uptake in macrophages; B. Effect of metformin intervention time on $^{18}$F-FDG uptake in macrophages; C.Effect of metformin intervention on $^{18}$F-FDG uptake in lung cancer cells.

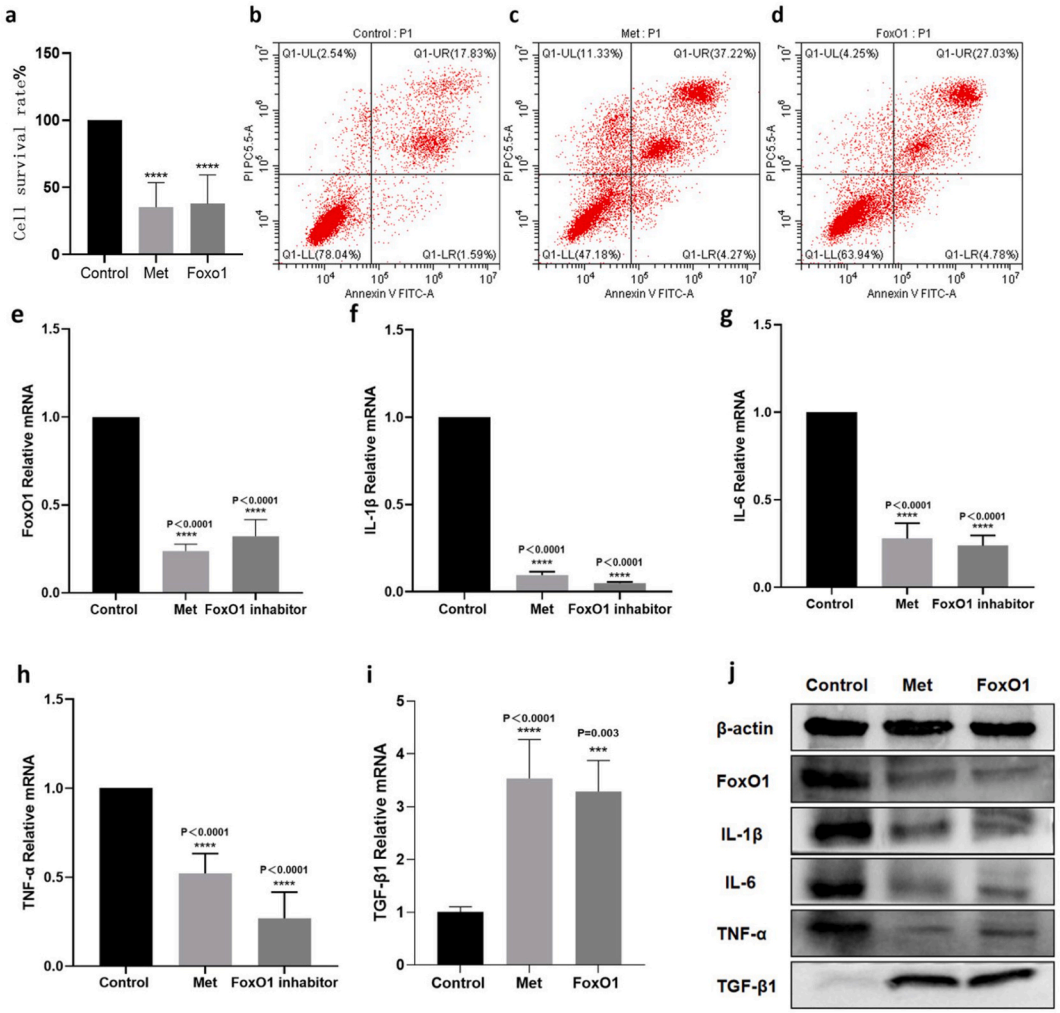


**Fig. 2.** A.CCK-8 to test the effect of metformin and FoxO 1 inhibitors on macrophage cell survival; B-D.The effect of metformin and FoxO1 inhibitors on apoptosis; E-J. Effect of metformin and FoxO1 inhibitors on the expression levels of FoxO1 and its downstream inflammatory factors IL-1β, IL-6, TNF-αand TGF-β1 in macrophages; ****, P < 0.0001.

the muscle tissue of FoxO1-KD mice exhibited a significant reduction in FoxO1 mRNA levels, showing a 58-fold decrease (1.026 ± 0.2686 vs 0.01743 ± 0.002958, P = 0.0047). Additionally, there was a 2-fold decrease in FoxO1 protein levels in muscle tissue of these mice (1.264 ± 0.0726 vs 0.6328 ± 0.0378, P = 0.0060), indicating a marked reduction in FOXO1 expression. Furthermore, after injection of an equivalent amount of turpentine in FoxO1-KD mice, $^{18}$F-FDG uptake was significantly decreased in FoxO1-KD mice compared to the normal $^{18}$F-FDG uptake in C57 mice (3.94 ± 0.374 vs 2.445 ± 0.435, P = 0.0286).

Interestingly, there was no significant difference in $^{18}$F-FDG uptake between FoxO1-KD mice and C57 mice treated with metformin. This finding suggests that metformin intervention could effectively reduce $^{18}$F-FDG uptake in inflammatory conditions through the modulation of FoxO1.

## 4. Discussion

The elevated uptake of $^{18}$F-FDG by pulmonary inflammatory lesions compromises the diagnostic and staging accuracy of $^{18}$F-FDG PET/CT in lung cancer. Current solutions to this challenge include specific probes like $^{68}$Ga-RGD, $^{11}$C-choline, and $^{18}$F-benzoic acid [17,18]. However, these probes are difficult to prepare, costly, and other drugs have limited applications. We propose leveraging metformin's anti-inflammatory effect to reduce lung inflammation's uptake of $^{18}$F-FDG and explore metformin's potential mechanisms. This approach could enhance the diagnostic accuracy of lung cancer and expand our understanding of metformin's anti-inflammatory mechanisms.

Following intervention with metformin and FoxO1 inhibitors, we observed a 4-fold and 3-fold reduction in FoxO1 expression in macrophages, respectively. The expression of downstream inflammatory factors IL-1β, IL-6, TNF-α by FoxO1 was reduced by 22-fold,

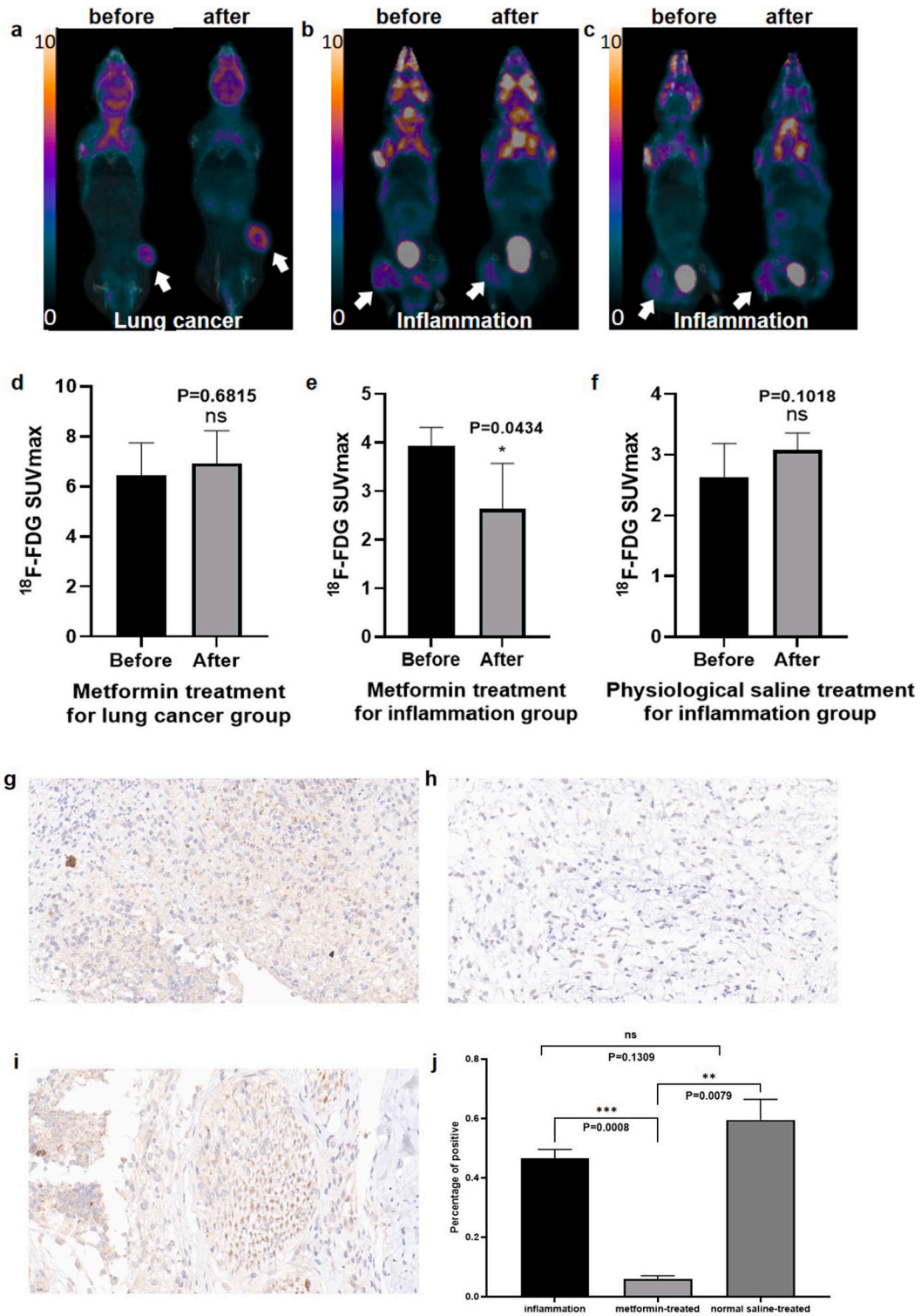


**Fig. 3.** A-C.The changes in $^{18}$F-FDG uptake before and after drug intervention (A、B for metformin intervention, C for physiological saline intervention); D-F.The comparison of $^{18}$F-FDG SUVmax before and after drug intervention in A-C mouse models; G-I.Immunohistochemical staining of FoxO1 at the inflammatory sites. G、H represents FoxO1 staining before and after metformin intervention, and I represents FoxO1 staining after saline intervention.(Picture scale bar is 20um); J.Comparison of immunohistochemistry between each group of mice.

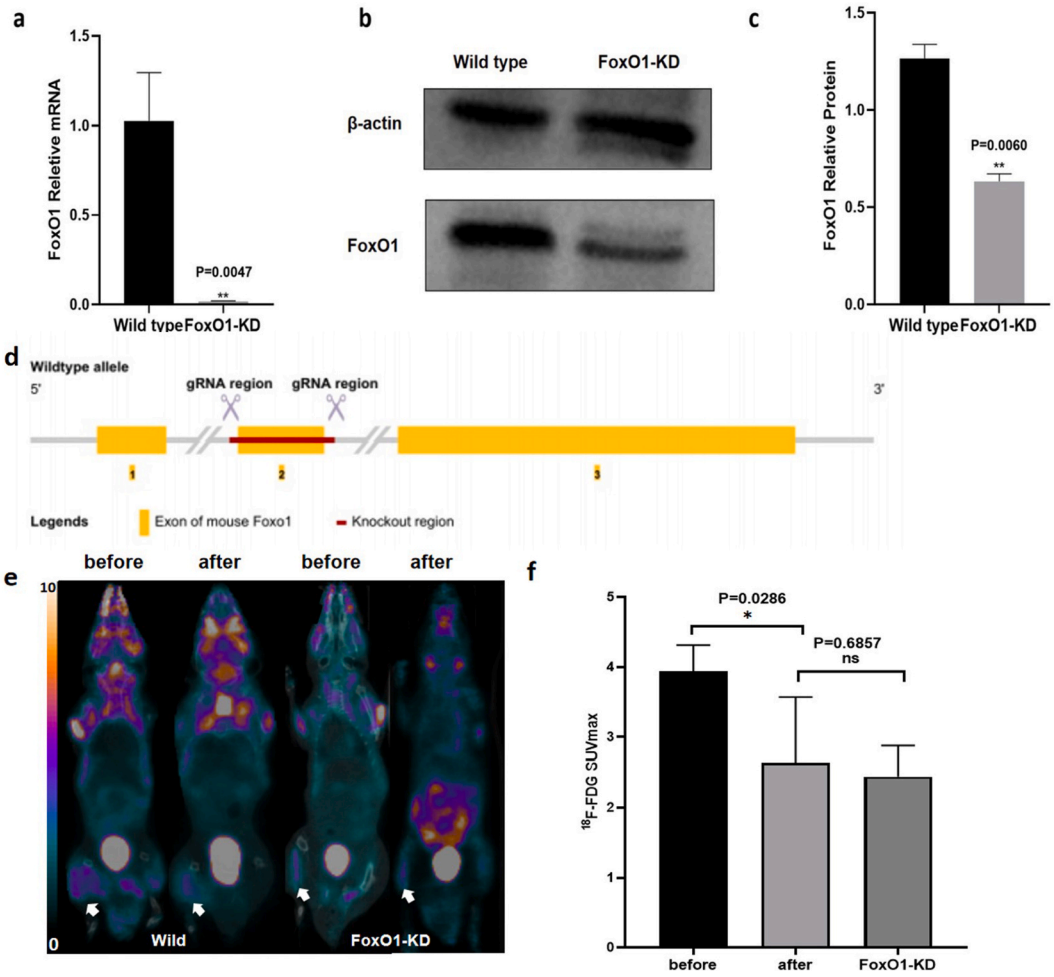


**Fig. 4.** A-C.FoxO1 expression levels in tissues from FoxO1-KD mice; D.Overview of the Targeting Strategy; E. Metformin intervention in inflammatory models of common C57 and FoxO1-KD mice: $^{18}$F-FDG PET/CT imaging.

10.6-fold, 3.7-fold, and 2-fold, 4-fold, 3.7-fold, respectively. These interventions significantly increased macrophage apoptosis and decreased inflammatory factors. Metformin intervention led to a 35 % reduction in macrophage uptake of $^{18}$F-FDG and a 34 % reduction in vivo inflammation of $^{18}$F-FDG uptake, without significantly affecting tumor $^{18}$F-FDG uptake.

Metformin's anti-inflammatory properties have been demonstrated in numerous studies [16,19,20]. It has been used to treat rheumatoid arthritis, significantly improving inflammation and patient quality of life. In keratinocyte HaCaT cells, metformin notably reduced inflammatory factor production [19]. A population-based case-control study of 36,702 psoriasis patients also indicated that long-term metformin use was associated with a reduced risk of psoriasis [20]. These clinical studies suggest metformin's anti-inflammatory effect and its potential to reduce FDG uptake. Due to the complexity of its anti-inflammatory mechanisms, we reviewed metformin's effects, highlighting the significant role of FoxO1 in inflammation, lipid metabolism, cell cycle regulation, development, apoptosis, and autophagy [21–25]. FoxO1 is closely associated with chronic metabolic inflammation, such as insulin resistance [23], and regulates NF-κB expression in liver and colorectal cancers [19,26,27]. Our study demonstrated that metformin can downregulate FoxO1 expression, regulate apoptosis, and inflammatory mediator expression in macrophages, thereby reducing inflammation's uptake of $^{18}$F-FDG. Metformin, a first-line drug for treating type 2 diabetes, is a low-cost, high-safety, non-hormonal drug, making it a promising strategy to reduce $^{18}$F-FDG uptake in pulmonary inflammatory lesions.

Pharmacological intervention studies on FDG uptake in pulmonary inflammation are scarce, with reported drugs mainly being steroids [28,29]. Zhao S et al. reported using high-dose methylprednisolone to pre-treat tumor and intramuscular granuloma rat models, significantly reducing FDG uptake in intramuscular granuloma [28]. A small clinical study of 17 cases used dexamethasone to reduce $^{18}$F-FDG uptake in lung inflammatory lesions [29]. However, these drugs have safety issues and can affect blood glucose levels [28,30,31], potentially leading to false negatives in tumors [32,33]. Our use of metformin showed no impact on lung cancer's uptake of $^{18}$F-FDG, primarily because metformin did not affect the key transporter protein GLUT1 involved in tumor FDG uptake [34–37].

The limitation of our study is that the effect of metformin on lung cancer and inflammation was only compared at a single time point before treatment, not dynamically. In our previous study on hepatocellular carcinoma, we found that 48 h of metformin

intervention was sufficient to affect FoxO1 expression [37]. Similarly, in vitro macrophage experiments demonstrated that 48 h was adequate. Therefore, imaging was conducted after a 48-h intervention period. The anti-inflammatory mechanism of metformin is complex, and while we confirmed the role of FoxO1 in FDG uptake by constructing a FoxO1-KD model, other molecular mechanisms still exist, including ATF3, SIRT1, PGC-1α, etc [14]. In conclusion, metformin's reduction of inflammatory $^{18}$F-FDG uptake through FoxO1 provides a feasible strategy to distinguish benign inflammation from malignancy, although further validation in other models and patients is needed.

We have linked metformin's anti-inflammatory effect with macrophages in inflammatory lesions. By exploring the mechanism of metformin in reducing macrophage uptake of $^{18}$F-FDG, we have provided a feasible strategy for clinically reducing false positives in $^{18}$F-FDG PET/CT.

## CRediT authorship contribution statement

**Yu yue Feng:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xin Xiang:** Writing – original draft, Formal analysis, Data curation. **Yu Weng:** Visualization, Methodology, Conceptualization. **Biao Xia:** Visualization. **Hong cheng Li:** Methodology. **Yue Li:** Software. **Lin jun Ju:** Software. **Fei Kang:** Writing – review & editing, Project administration. **Hua Pang:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Zheng jie Wang:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

## Data availability statement format guidelines

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

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## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Zhengjie Wang reports financial support was provided by Joint Project of Chongqing Health Commission and Science and Technoloogy Bureau. Zhengjie Wang reports financial support was provided by General program of Chongqing Natural Science Foundation. 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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