*Sensors and Materials*, Vol. 35, No. 6 (2023) 1849–1857
MYU Tokyo



S & M 3293

# Metformin Intervention in Rats with Acute Gouty Arthritis

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(Received January 7, 2023; accepted May 9, 2023)

***Keywords:*** gout, metformin, FFA, MSU

The purpose of this study was to determine the intervention effect of metformin on rats with acute gout and the influence of serum free fatty acids (FFAs). Twenty-one Wistar rats were randomly divided into the 200 mg/kg metformin group, 300 mg/kg metformin group, and control group. After five days of administration, sodium urate crystals were injected into the metatarpal joints of the rats to establish the model. The volume change of the metatarpal joints of the rats was measured and we used a biochemical sensor to determine the FFA concentration in serum. Metformin administered at 300 mg/kg body weight showed good anti-inflammatory and anti-swelling effects within 24 h after modeling, and reduced the swelling rate of the ankle joint. Compared with the control group, the ankle swelling rate of the 300 mg/kg metformin group decreased 36 h after modeling and was lower than that of the 200 mg/kg metformin group 36, 72, and 96 h after modeling ($P < 0.05$). The serum FFA concentration in the 300 mg/kg metformin group significantly decreased compared with that in the control group, and the difference was statistically significant ($P < 0.05$). In conclusion, 300 mg/kg metformin can inhibit the joint swelling of rats with acute gouty arthritis, and the effect is better than that of 200 mg/kg metformin. The possible mechanism of metformin in the treatment of acute gouty arthritis in rats is as follows: by reducing the FFA concentration in serum, metformin interferes with the acute attack of gouty arthritis. A possible mechanism of the effect of metformin in treating acute gouty arthritis in rats is the intervention of the acute onset of gouty arthritis by inhibiting the increase in serum FFA concentration.

## 1. Introduction

Gout refers to acute and chronic inflammation and tissue destruction caused by the precipitation of monosodium urate (MSU) in subcutaneous tissues such as joints and kidneys. It is a serious disease caused by the dysfunction of purine metabolism and urate secretion.(1) The current prevalences of gout are 0.9% in France, 1.4% in Germany, and 1.9% in the United Kingdom.(2) With the improvement of living standards, the prevalence of gout in the Chinese population has increased significantly, ranging from 0.5 to 5%, mainly as a result of the change in diet structure.(3) However, the current understanding of the pathogenesis of gout is not clearly improved.

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https://doi.org/10.18494/SAM4311

ISSN 0914-4935 

Most of the research results show that the pathogenesis of gout is related to the metabolism, inflammation, and immunity of patients. The end product of purine metabolism is uric acid, and the elevation of serum uric acid concentration is considered to be the cause of gout attacks. Scientific studies have shown that interleukin-1 (IL-1), toll-like immune receptor (TLR), neutrophil alkaline phosphatase 3 (NLRP3), and urate crystals are involved in the development and progression of gouty arthritis.[4] TLR can be activated by free fatty acids (FFAs), using the interaction between urate crystals and immune cells, and can be transcribed and translated into pre-IL-1 and processed to produce bioactive IL-1, and thus produce various inflammatory responses.[4] Therefore, drugs that reduce FFA concentration in serum may have an intervention effect on the onset of acute gout.

Metformin is an oral hypoglycemic agent widely used in the treatment of type 2 diabetes mellitus, and recent studies have shown evidence of the multipotency of metformin, including an interesting anti-inflammatory activity. Since the drug improves hyperglycemia, insulin resistance, and lipid profiles, it may itself reduce chronic inflammation.[5] Some studies have shown that metformin has some preventive effect on gout,[6] and we speculate that the mechanism may be related to the reduction in FFA concentration in serum. Metformin can reduce FFA concentration in serum; therefore, we used metformin to investigate its intervention in the acute attacks of gout. We used a biochemical sensor to detect the FFA concentration in rat serum after metformin administration to elucidate its possible mechanism of action in gout.

## 2. Materials and Methods

### 2.1 Materials

The following were available in the laboratory: male Wistar rats (<6 w, 120–150 g), metformin (0.25 g), sodium urate (1 g), normal saline (500 ml), plastic beakers (25 and 50 ml), syringes (10, 2.5, and 1 ml), gavage injection (No. 6), heparin sodium extraction vessel (5 ml), venous needle (No. 5.5), high-speed refrigerated centrifuge (Hitachi High-tech Co., Ltd., CF15RN), laboratory ultrapure water machine (Zerab Instrument Technology Co., Ltd., Dura12), electronic balance instrument (Ohaus Instrumentation Co., Ltd., Scout SE-SE602F), medical surgical scissors, refrigerator (Qingdao Haier Co., Ltd., BCD-248WTPM), gavage needle, and foot measuring instruments.

### 2.2 Methods

#### 2.2.1 Establishment of acute gouty arthritis (AGA) animal model

After positioning the rat, puncture locations were selected on the lateral and posterior sides of the right ankle, and the synovial joint cavity was placed at a 45° angle between the needle and the ankle. The needle was tilted upward, and a 100 μL suspension of MSU (25 mg/mL) was injected into the joint cavity to obtain the acute arthritis model.

### 2.2.2 Experimental procedure

The test rats were weighed and randomly divided into the 200 mg/kg metformin group, 300 mg/kg metformin group, and control group with prophylactic gavage for 5 days. On the sixth day of metformin administration, the knee joints of three rats were first injected with MSU crystals for external knee joint modeling, as above. The expression, hair color, feeding, excretion, and limb movement of all the rats were observed before and after modeling.

Before modeling, the rats in each group were marked with a black marker about 0.4–0.5 cm above the right ankle. The glass container method was used for measurement. The toe volume was measured at the 4, 12, 24, 36, and 48th hours after modeling, by the same method as above. To reduce operating errors, each measurement was performed twice.

Swelling rate (%) = (toe volume after modeling − toe volume before modeling)/toe volume before modeling × 100 %.

We used the colorimetric sensor system developed by Arslan *et al.*[7] to determine the FFA concentration in serum. The reaction process in the sensor was as follows: FFAs react with CoA to form acetyl-CoA oxidase (ACO). Acetyl-CoA generates $H_2O_2$ under the action of ACO and then generates a colored substrate under the action of peroxidase (POD) through chromogens. The analytical instrument was an automatic biochemical analyzer. Quality control was carried out in accordance with the instructions for the quality control products. Quality control experiments were conducted once a day. The kit was purchased from Meikang Biotechnology Co., Ltd. The specific experimental procedures were carried out in accordance with the instructions.[8]

## 3. Results

### 3.1 General indicator observation

The hair color of the rats in the control group was white and glossy, and they behaved normally and ate normally. The rats in the 200 mg/kg metformin group had normal hair color, lighter body weight, and mild diarrhea, and consumed less food and water than those in the control group. Rats in the 300 mg/kg metformin group had normal hair color, normal movement, and diarrhea, and consumed less food and showed slower weight gain than those in the control group.

### 3.2 Change in swelling rate of ankle joint in each group

At the initial stage of inflammation, there was a significant difference in ankle swelling rate between the control group and the 200 mg/kg metformin group ($P < 0.05$). The ankle swelling rate in the control group reached its peak at 36 h, and the metformin groups also reached their peaks at 36 h after modeling. The joint swelling rate was significantly lower in all metformin groups than in the control group ($P < 0.01$). After modeling, the swelling rates were significantly lower in the 200 and 300 mg/kg metformin groups than in the control group ($P < 0.01$). The

metformin groups showed reduced foot swelling after 36 h, and the foot swelling of the control group was less than those of the metformin groups (as shown in Fig. 1 and Tables 1–3).

As shown in Table 1, the nonparametric test was used to study the differences among the control group, 200 mg/kg metformin group, and 300 mg/kg metformin group over time. Kruskal–Wallis test statistics was used for the analysis. For all groups, all the samples at different times showed significant results ($P < 0.05$), indicating that the samples at different times showed differences for the above three groups.

### 3.3 Kruskal–Wallis H test of serum FFA concentration in different groups

The Kruskal–Wallis H test was used to compare the distribution difference of FFA concentration among the 200 mg/kg metformin group, 300 mg/kg metformin group, and control group. According to the histogram, the distribution shapes of FFA concentration in all the groups were basically the same. The distributions of FFA concentration in all the groups were not identical, and the differences were statistically significant ($H = 7.871$, $P < 0.05$). The median FFA concentrations were 0.59 ($n = 7$) in the control group (as shown in Fig.2), 0.44 ($n = 7$) in the 200 mg/kg metformin group, and 0.37 ($n = 7$) in the 300 mg/kg metformin group. From the above results, the pair comparison after using the Bonferroni method to adjust the significance

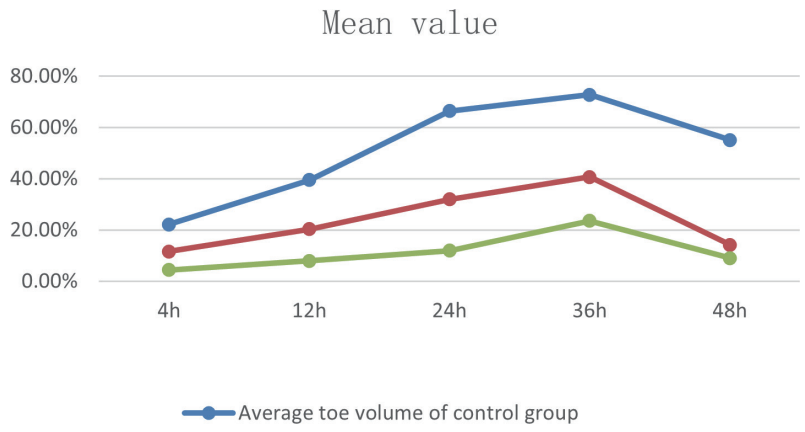


Fig. 1. (Color online) Plantar swelling trend.

Table 1
Nonparametric test analysis results.

| | Median number M (P25, P75) | | | | | *H* | *P* |
|---|---|---|---|---|---|---|---|
| | 12 h ($n = 7$) | 24 h ($n = 7$) | 36 h ($n = 7$) | 48 h ($n = 7$) | 4 h ($n = 7$) | | |
| Control group | 0.56 (0.40, 0.68) | 0.80 (0.72, 1.11) | 0.94 (0.76, 1.19) | 0.49 (0.39, 1.17) | 0.26 (0.24, 0.37) | 21.42 | 0.00** |
| 200 mg/kg metformin group | 0.24 (0.23, 0.29) | 0.41 (0.38, 0.45) | 0.52 (0.50, 0.56) | 0.18 (0.14, 0.19) | 0.15 (0.12, 0.16) | 30.53 | 0.00** |
| 300 mg/kg metformin group | 0.08 (0.08, 0.12) | 0.14 (0.12, 0.18) | 0.30 (0.24, 0.33) | 0.11 (0.09, 0.13) | 0.04 (0.04, 0.07) | 26.49 | 0.00** |

Note: $P < 0.03$, $^{**}P < 0.01$

Table 2
FFA concentration in each group.

| Summary of independent sample Kruskal–Wallis test | |
|---|---|
| Total *N* | 21 |
| Statistics of inspection | 518 |
| Degree of freedom | 7.871[a] |
| Progressive significance (two-sided test) | 0.2 |

[a]Inspection statistics are adjusted for the binding value.

Table 3
Pairwise comparison of groups.

| Sample 1–Sample 2 | Statistics of inspection | Standard error | Standard test statistics | Significance | Adj. significance |
|---|---|---|---|---|---|
| 300 mg/kg metformin group–200 mg/kg metformin group | 4.214 | 3.314 | 1.271 | 0.204 | 0.611 |
| 300 mg/kg metformin group–control group | 9.286 | 3.314 | 2.802 | 0.005 | 0.015 |
| 200 mg/kg metformin group–control group | 5.071 | 3.314 | 1.530 | 0.126 | 0.378 |

Note: Each row shows results of testing the null hypothesis that sample 1 has the same distribution as sample 2.
Progressive significance was shown (two-sided test). The significance level was 0.05.
Significance values were adjusted by Bonferroni correction for a number of tests.

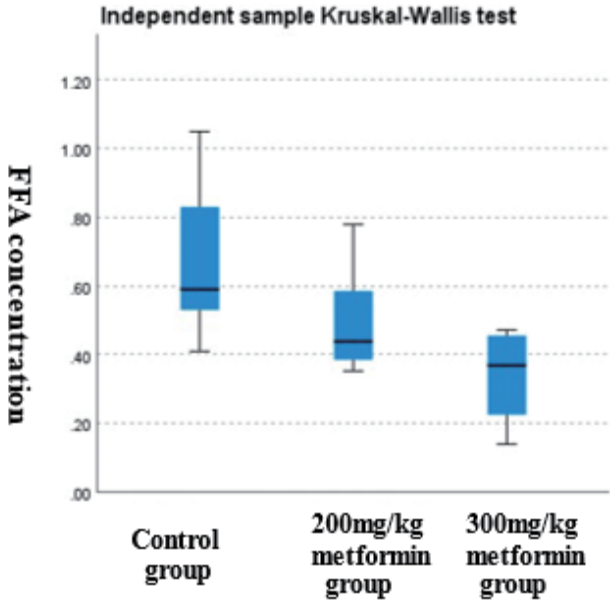


Fig. 2. (Color online) Median and distribution of FFAs in each group.

level revealed that the distributions of FFAs in the 300 mg/kg metformin group and control group were statistically significant, whereas the differences between the other groups were not statistically significant.

## 4. Discussion

Gout is one of the common diseases in daily life. With the development of society and the improvement of people's living standards, the incidence of acute gout arthritis has increased significantly in recent years.[9] Its pathogenesis is still unclear, but in recent years, studies have suggested that the causes of acute gout arthritis include the increase in serum FFA concentration and the deposition of urate crystals.[10] Some studies suggest that metformin, commonly used to treat type 2 diabetes, reduces cellular inflammation caused by MSU crystals and may reduce the burden of gout attacks.[11] Metformin has been shown to reduce FFA concentration, so it is possible that metformin may prevent acute gout attacks by lowering FFA concentration. We used a biochemical sensor to determine the serum FFA concentration in rats, combined with the preventive effect of metformin on the acute onset of gout, to elucidate the possible mechanism of metformin.

Gout is modeled by inserting a syringe into the ankle cavity from the lateral side of the right ankle at an angle of 45° and injecting 0.2 mL of MSU suspension (2.5 g/100 mL).[12] A successful injection is indicated by a contralateral bulge of the joint capsule. However, this method has shortcomings, namely, it is easy to damage local tendons and nerves, cause mechanical damage, and interfere with the determination of the local joint dysfunction of the model. Therefore, the skill level of researchers should be high. After searching the literature, the solution is as follows: in addition to improving the skill level of researchers, the modeling method of Xu *et al.*, that is, using the minimally invasive embedding of saturated MSU to induce persistent gout arthritis in the improved rat model,[13] can be used for modeling if the experimental conditions permit. This method causes no mechanical damage to the local tendons and nerves of rats.[11]

In this experiment, urate was injected into the foot of the rat during modeling. However, it was difficult to inject the urate suspension into the foot of the rat. Alghadir *et al.* studied the therapeutic effects of curcumin and ion electrophoresis on claw edema and hematological reaction in the collagen-induced arthritis rat model.[14] In their study, 4 mg/kg collagen suspension was injected intradermally into multiple areas around the ankle joint and the base of the tail. When a booster dose was given one week after the initial inoculation, plantar swelling was evident, and it was easier to inject collagen into the ankle than urate. The injection can also provide a strong reference for future research.

FFAs are composed of oleic acid, palmitic acid, and linoleic acid, which are mostly combined with albumin and exist in the blood.[15] The risk factors for gout (such as overeating and drinking) are associated with the increase in serum FFA concentration. Some studies have shown that in a mouse model of gout, arthritis was observed only when the mice were injected with MSU crystals and long-chain FFAs simultaneously, but not with only one of the two. It is speculated that FFAs react with sodium urate to induce gout inflammation.[16] Therefore, in this experiment, the FFA concentration in the serum of rats in the 200 mg/kg metformin group, 300 mg/kg metformin group, and control group were determined and statistically analyzed as a preliminary test to explore the possible relationship of FFA concentration with acute gout attack. The results showed that the serum FFA concentration was significantly lower in the metformin group than in the control group, suggesting that FFA may be one of the factors affecting the onset of gout.

Sensors for the detection of FFAs have been studied. In 2005, Bartolome *et al.* developed the use of an FFA-binding protein labeled with acrylamide intestinal fatty-acid-binding protein.[17] It was modified with a ruthenium metal-ligand complex to create a sensor for FFA determination that has the advantage of low cost and can be used without sacrificing measurement quality. The disadvantage is that it is relatively difficult to obtain acrylamide-conjugated intestinal fatty-acid-binding protein. Tang *et al.* developed a method for the fast detection of fatty acids using terahertz time-domain spectroscopy combined with metamaterial terahertz sensors. The disadvantage is that resolution and sensitivity need to be further improved, and new methods need to be developed in subsequent studies to identify fatty acids with different saturations and conformations.[18] Yang *et al.* genetically incorporated a synthetic fluorescent amino acid [L-(7-hydroxycoumarin-4-yl) ethyl glycine, Cou] into a fatty-acid-binding protein, and a fluorescence sensor with an open signal in the presence of FFAs was developed[19] with the advantage of being able to detect FFAs under close to physiological conditions (pH 7.8) and exhibit high specificity and sensitivity. The disadvantage is that the materials for this sensor are expensive and not readily available. On the basis of the colorimetric sensor system developed by Arslan *et al.*[7] and the principle of enzyme reaction, we developed a biochemical sensor for the quantitative determination of FFAs in rat serum. Our method has the advantages of speed, convenience, and low cost.

The analysis of excess weight results showed that the weights of the control group and 200 mg/kg group increased normally, and the weights of these two groups increased more than that of the 300 mg/kg group. Shin *et al.* showed that metformin treatment at a concentration of 300 mg/kg can significantly prevent olanzapine-induced insulin resistance.[20] In this study, metformin was administered at a concentration of 300 mg/kg. When all the compounds were suspended in 0.5% methylcellulose, the rats slowly gained weight. Studies have shown that the anti-diabetic, appetite-suppressive, and weight-reducing effects of metformin have been confirmed in obese animals. While the exact mechanism is still unknown, these findings raise the possibility that the metabolic benefits achieved with metformin, particularly weight loss effects, may be mediated in part by restoring HPA function and/or integrity. The direct effect of metformin on the HPA axis seems plausible because of its ability to cross the blood-brain barrier with ease. In this experiment, the slow weight gain of rats in the metformin groups is probably due to the direct effect of metformin on the HPA axis. Although this study provides a certain basis for metformin to reduce the incidence of gout attacks, there are still many problems to be solved, and the mechanism behind this action needs to be elucidated in the future.

## 5. Conclusions

In this study, metformin was selected as a lipid-lowering drug to study its intervention effect on gout. The experimental results preliminarily confirmed that metformin can reduce the incidence of gout attacks to a certain extent, but further verification is still needed. The sensor developed in this study can also be used to monitor inflammation in gout patients.

## Acknowledgments

This work was supported by the Fujian Province Young and Middle-aged Teacher Education and Research Project (JAT190572) and Putian University Teaching Reform Research Project (JG202178).

## References

1 T. Bardin and P. Richette: Lancet Rheumatol. **4** (2022) E7. https://doi.org/10.1016/S2665-9913(21)00358-1
2 M. Dehlin, L. Jacobsson, and E. Roddy: Nat. Rev. Rheumatol. **16** (2020) 380. https://www.nature.com/articles/s41584-020-0441-1
3 J. Huang, Z.F. Ma, Y. Tian, and Y. Y. Lee: SN Compr. Clin. Med. **2** (2020) 1593. https://link.springer.com/article/10.1007/s42399-020-00416-8
4 A. K. So and F. Martinon: Nat. Rev. Rheumatol. **13** (2017) 639. https://www.nature.com/articles/nrrheum.2017.155
5 Y. Saisho: Endocr. Metab. Immune Disord. Drug Targets **15** (2015) 196. https://doi.org/10.2174/1871530315666150316124019
6 N. Vazirpanah, A. Ottria, M. Linden, C. Wichers, M. Schuiveling, E. Lochem, A. Phipps-Green, T. Merriman, M. Zimmermann, M. Jansen, T. Radstake, and J. Broen: Ann. Rheum. Dis. **78** (2019) 663. https://doi.org/10.1136/annrheumdis-2018-214656
7 M. Arslan, M. Zareef, H. E. Tahir, Z. Xiaodong, A. Rakha, S. Ali, J. Shi, and Z. Xiaobo: Spectrochim. Acta, Part A **292** (2023) 122359. https://doi.org/10.1016/j.saa.2023.122359
8 L. Pei, L.F. Xie, J.Y. Wu, H. Zhang, and X. W. Zhang: Clin. Rheumatol. **39** (2020) 1251. https://doi.org/10.1007/s10067-019-04903-9
9 Q. H. Gao, X. Y. Cheng, T. R. Merriman, C. Wang, L. L. Cui, H. Zhang, W. Y. Sun, J. Wang, F. Y. Wang, C. G. Li, and J. Lu: Joint Bone Spine **88** (2020) 6. https://doi.org/10.1016/j.jbspin.2020.09.010
10 L. A. B. Joosten, M. G. Netea, E. Mylona, M. I. Koenders, R. K. Subbarao Malireddi, M. Oosting, R. Stienstra, F. L. van de Veerdonk, A. F. Stalenhoef, E. J. Giamarellos-Bourboulis, T.-D. Kanneganti, and J. W. M. van der Meer: Arthritis Rheum. **62** (2010) 3237. https://doi.org/10.1002/art.27667
11 T. R. Mikuls: N. Engl. J. Med. **387** (2022) 1877. https://doi.org/10.1056/NEJMcp2203385
12 Y. Liu, X. Zhu, S. Ji, Z. Huang, Y. Zang, Y. Ding, J. Zhang, and Z. Ding: Drug Deliv. **29** (2022) 2984. https://doi.org/10.1080/10717544.2022.2122632
13 H. L. Xu, S. K. Li, X. A. Xue, Z. Y. Chen, and Y. Hua: Biomed. Res. Int. **2021** (2021) 6641701. https://doi.org/10.1155/2021/6641701
14 A. Alghadir, M. Miraj, and S. Ali: Evid Based Complement Alternat Med. **2020** (2020) 4606520. https://doi.org/10.1155/2020/4606520
15 G. Boden: Endocrinol Metab. Clin. North Am. **37** (2008) 635. https://doi.org/10.1016/j.ecl.2008.06.007
16 E. J. Giamarellos-Bourboulis, M. Mouktaroudi, E. Bodar, J. van der Ven, B.-J. Kullberg, M. G. Netea, and J. W. M. van der Meer: Ann. Rheum. Dis. **68** (2009) 273. https://doi.org/10.1136/ard.2007.082222
17 A. Bartolome, C. Bardliving, G. Rao, and L. Tolosa: Anal. Biochem. **345** (2005) 133. https://doi.org/10.1016/j.ab.2005.07.030
18 M. Tang, L. Xia, D. Wei, S. Yan, M. Zhang, Z. Yang, H. Wang, C. Du, and H. L. Cui: Spectrochim. Acta, Part A **228** (2020) 117736. https://doi.org/10.1016/j.saa.2019.117736
19 K. Yang, M. Yu, X. Zhu, Y. Xia, F. Li, Y. Li, X. Liu, and J. Wang: J. Mol. Biol. **434** (2022) 167498. https://doi.org/10.1016/j.jmb.2022.167498
20 A. C. Shin, P. Balasubramanian, P. Suryadevara, J. Zyskowski, T. H. Herdt, S. M. J. MohanKumar, and P. S. MohanKumar: Int. J. Obes. **45** (2021) 383. https://doi.org/10.1038/s41366-020-00688-z

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