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# Factors influencing shoulder stiffness after open reduction and internal fixation of proximal humeral fractures

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## ABSTRACT

**Objectives:** The study aimed to investigate the factors associated with shoulder stiffness following open reduction and internal fixation (ORIF) of proximal humeral fractures.

**Patients and methods:** The retrospective study included a total of 151 patients who underwent ORIF of proximal humeral fractures between January 2016 and May 2021. Based on their shoulder joint motion at the latest follow-up, the patients were divided into two groups. The stiffness group (n=32; 8 males, 24 females; mean age: 62.4±9.3 years; range, 31 to 79 years), exhibited restricted shoulder forward flexion (<120°), limited arm lateral external rotation (<30°), and reduced back internal rotation below the L3 level. The remaining patients were included in the non-stiffness group (n=119; 52 males, 67 females; mean age: 56.4±13.4 years; range, 18 to 90 years). Various factors were examined to evaluate the association with shoulder stiffness following ORIF of proximal humeral fractures by multivariate unconditional logistic regression models.

**Results:** The mean follow-up duration was 31.8±12.6 (range, 12 to 68) months. Based on the results of the multivariate regression analysis, it was found that high-energy injuries [compared to low-energy injuries; adjusted odds ratio (aOR)=7.706, 95% confidence interval (CI): 3.564-15.579, p<0.001], a time from injury to surgery longer than one week (compared to a time from injury to surgery equal to or less than one week; aOR=5.275, 95% CI: 1.7321-9.472, p=0.031), and a body mass index (BMI) >24.0 kg/m$^2$ (compared to a BMI between 18.5 and 24.0 kg/m$^2$; aOR=4.427, 95% CI: 1.671-11.722, p=0.023) were identified as risk factors for shoulder stiffness following ORIF of proximal humeral fractures.

**Conclusion:** High-energy injury, time from injury to surgery longer than one week, and BMI >24.0 kg/m$^2$ were identified as independent risk factors for shoulder stiffness after proximal humeral fracture surgery, which should be treated with caution in clinical treatment.

***Keywords:*** Fracture internal fixation, function, proximal humerus fracture, range of motion, risk factors, shoulder stiffness.

Proximal humeral fractures account for 4 to 5% of all fractures and are one of the most common clinical fractures.[1] Among elderly patients, it is the third most common osteoporotic fracture after wrist and hip fractures.[2,3] For Neer type 1 and 2 proximal humeral fractures, conservative treatment can mostly achieve satisfactory results, and surgical treatment is still the preferred treatment method for complex Neer type 3 and 4 proximal humeral fractures.[4,5] The treatment of proximal humeral fractures with locking plates preserves bone mass and the possibility of anatomical healing. Previous studies have also reported good clinical outcomes of open reduction and internal fixation (ORIF). However, there is a high probability of postoperative complications, such

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as shoulder stiffness, subacromial impact, fracture redisplacement, screw cutting, intra-articular screw penetration, and ischemic necrosis of the humeral head.[6-8]

Shoulder stiffness is a frequent complication after proximal humeral fractures treated with surgery.[9] Accordingly, it is crucial to prevent shoulder stiffness in the treatment of proximal humeral fractures. Currently, there is no consensus on the diagnostic criteria for shoulder stiffness, which is often diagnosed based on limited active and passive movement of the shoulder, including forward flexion, lateral rotation, and posterior internal rotation.[10] In clinical practice, it is usually affected by multiple factors, and some patients cannot recover to a satisfactory range of motion of the shoulder after surgery, which greatly affects the quality of life of the patients. A previous study analyzed the factors influencing shoulder stiffness occurring secondary to rotator cuff tears.[11] Nevertheless, the factors influencing postoperative traumatic shoulder stiffness in patients with proximal humeral fractures treated with open reduction and locking plate internal fixation have not been fully reported.

Hence, this study aimed to assess patients with shoulder stiffness after fracture surgery to compare patients according to the range of motion of the shoulder and investigate demographic, injury, and treatment factors potentially associated with the development of shoulder stiffness.

## PATIENTS AND METHODS

Consecutive patients diagnosed with proximal humeral fractures who underwent ORIF at the Third Hospital of Baotou City, Department of Orthopaedics between January 2016 and May 2021 were retrospectively reviewed. Inclusion criteria were as follows: *(i)* patients with proximal humeral fracture who were diagnosed through shoulder anteroposterior and transthoracic radiographs, computed tomography plain scan and iterative reconstruction; *(ii)* age ≥18 years to exclude the possibility of epiphyseal injury; *(iii)* patients with proximal humeral fractures who underwent ORIF with locking plates; *(iv)* patients with complete demographic information, surgical data, and follow-up results; *(v)* a clinical diagnosis of shoulder stiffness. Exclusion criteria were as follows: *(i)* patients with pathologic fractures, rotator cuff injuries the diagnosis was made by magnetic resonance imaging combined with intraoperative exploration, or a preinjury diagnosis of frozen shoulder; *(ii)* patients who lacked complete case data or were unable to undergo follow-up; *(iii)* patients with open fractures, old fractures, or fracture nonunion undergoing reoperation; *(iv)* patients with proximal humeral fractures who received other surgical treatments; *(v)* patients with severe underlying diseases, such as cardiovascular and cerebrovascular diseases, who could not tolerate surgery; *(vi)* patients with combined traumatic brain injury and brachial plexus injury; *(vii)* patients with severe cognitive impairment or mental disorders. We identified 172 patients with proximal humeral fractures who underwent ORIF over a five-year period. Following a further review of the patients, 21 patients were excluded, including five preinjury diagnoses of frozen shoulder, three patients younger than 18 years, two patients with conservative treatment, six patients with rotator cuff injuries, and five with incomplete medical records (Figure 1). Finally, 151 patients with shoulder stiffness were included in the study.

According to the forward flexion and rotation function at the last follow-up, 32 patients (8 males, 24 females; mean age: 62.4±9.3 years; range, 31 to 79 years) with shoulder forward flexion <120°, arm lateral external rotation <30°, and back internal rotation lower than the L3 level were classified as the stiffness group, and the above three conditions had to be satisfied simultaneously. The remaining 119 patients (52 males, 67 females; mean age: 56.4±13.4 years; range, 18 to 90 years) were included in the non-stiffness group. The relevant influencing factors that could cause postoperative shoulder stiffness included in this study were age, sex, body mass index (BMI), tobacco use, diseased side, injury mechanism, Neer classification of fractures, the AO classification of fractures, existence of additional fracture, time from injury to surgery, operation duration, intraoperative blood loss, surgical removal of internal fixation, the score of American Shoulder and Elbow Surgeons (ASES), the score of University of California Los Angeles (UCLA), and dominant hand.

### Surgical technique

All operations were performed by our senior chief physician, senior attending physician, and resident physician. All patients underwent ORIF with locking plates, and all fractures achieved anatomical reduction. After successful anesthesia, the patient was placed in the beach-chair position and operated on through the pectoralis major-deltoid muscle space approach. Through blunt separation of the pectoralis major muscle and deltoid muscle and protection of the cephalic vein, the proximal fracture site was exposed, the blood clots around the fracture were cleaned and washed, and Kirschner wires were

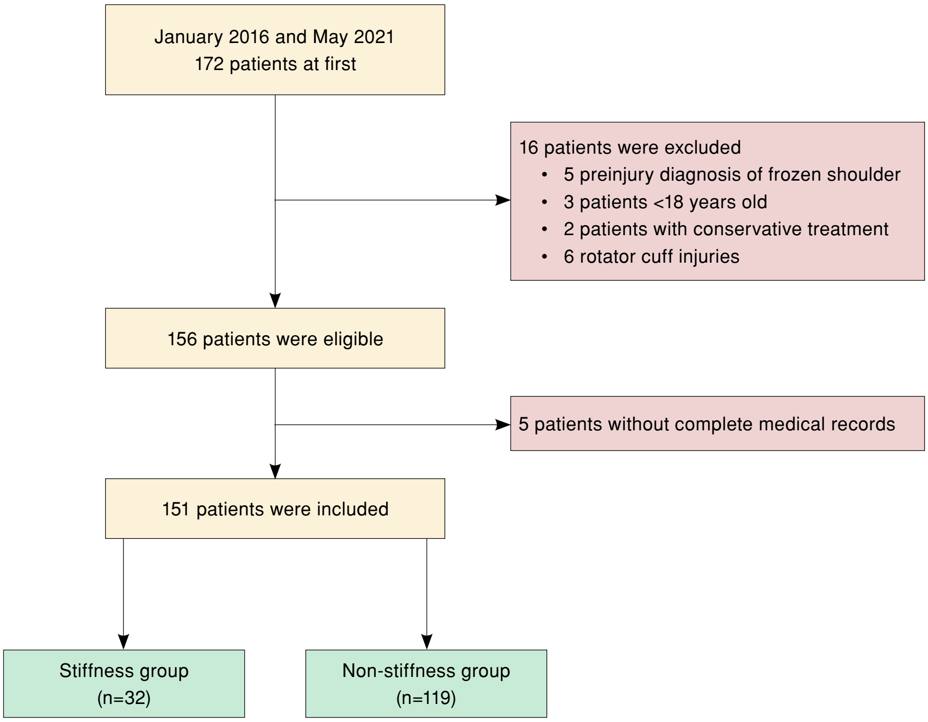


**FIGURE 1.** Selection of patients for the study.

inserted to reposition the fracture. After satisfactory repositioning under direct vision, Kirschner wires were temporarily fixed. After C-arm fluoroscopy confirmed the anatomical reduction of the fracture, a locking plate was placed outside the tendon of the long head of the biceps and approximately 0.5 cm below the top of the great tubercle. The C-arm fluoroscopy again showed that the length and position of each locking screw were appropriate, and after confirming good movement of the shoulder, the wound was washed and sutured.

### Postoperative rehabilitation and follow-up examination

Rehabilitation training was started as soon as possible after surgery. Six types of exercises were performed by the patients: *(i)* shoulder braking, *(ii)* active range of motion exercises, *(iii)* shoulder passive range of motion training, *(iv)* pendulum practice, *(v)* deltoid muscle isometric contraction training, and *(vi)* active activities of the scapula. For shoulder braking, the patients wore a shoulder arm sling, which could not be removed except for rehabilitation training. Active range of motion exercises included hand and wrist flexion and extension, forearm rotation, elbow flexion and extension, and other active activities. Shoulder passive range of motion training included shoulder forward flexion, abduction, lateral external rotation, external stand external rotation, and external stand internal rotation. Active activities of the scapula included shrugging the shoulders, lowering the shoulders, and expanding the chest. Outpatient follow-up examinations were conducted at one, two, three, six, and 12 months after surgery to adjust and guide rehabilitation training based on individual patient recovery. Passive training was performed gently, gradually, and not too forcefully. In addition, the follow-up patients were conducted based on their individual situations.

### Statistical analysis

Statistical analysis was performed using IBM SPSS version 25.0 software (IBM Corp., Armonk, NY, USA). Whether the data conformed to normal distribution was evaluated through the Shapiro-Wilk test, and the homogeneity of variance was assessed using dependent t-test. Continuous variables were presented as mean ± standard deviation (SD). The independent sample t-tests were used for intergroup comparisons. Count data (age, sex, BMI, tobacco use, diseased side, injury mechanism, Neer classification of fractures, AO classification of fractures, existence of additional fracture, time from injury to surgery,

**TABLE I**
Single factor analysis results of patients with shoulder stiffness

| Characteristics | Stiffness group (n=32) | Non-stiffness group (n=119) | OR | 95% CI | *p* |
|---|---|---|---|---|---|
| Age (year) | | | | | |
| ≤30 | 0 | 9 | - | - | 0.952 |
| 31~40 | 3 | 8 | 1.875 | 0.328~9.197 | 0.945 |
| 41~50 | 3 | 16 | 0.938 | 0.328~9.197 | 0.962 |
| 51~60 | 8 | 31 | 1.290 | 0.328~9.197 | 0.945 |
| 61~70 | 13 | 27 | 2.40 | 0.328~9.197 | 0.939 |
| ≥70 | 5 | 28 | 1.000 | | |
| Sex | | | | | |
| Male | 8 | 52 | 1.000 | | |
| Female | 24 | 67 | 0.429 | 0.178-1.034 | 0.060 |
| Diseased side | | | | | |
| Left | 13 | 57 | 1.000 | | |
| Right | 19 | 62 | 0.871 | 0.183-1.486 | 0.290 |
| Dominant hand | | | | | |
| Yes | 12 | 58 | 0.586 | 0.255-1.344 | 0.207 |
| No | 20 | 61 | 1.000 | | |
| Injury mechanism | | | | | |
| High-energy | 23 | 35 | 6.134 | 2.577-14.493 | <0.001* |
| Low-energy | 9 | 84 | 1.000 | | |
| Fracture type (AO classification) | | | | | |
| C1 | 0 | 13 | - | | 0.974 |
| B1.1 | 0 | 19 | - | | 0.971 |
| B1.2 | 21 | 71 | 0.430 | 0.173-1.068 | 0.943 |
| C3 | 11 | 16 | 1.000 | | |
| Fracture type (Neer classification) | | | | | |
| 3 | 21 | 91 | 0.587 | 0.253-1.365 | 0.216 |
| 4 | 11 | 28 | 1.000 | | |
| Existence of additional fracture | | | | | |
| Yes | 5 | 9 | 0.736 | 0.455-1.744 | 0.271 |
| No | 27 | 110 | 1.000 | | |
| Time from injury to surgery (week) | | | | | |
| ≤1 | 18 | 100 | 1.000 | | |
| >1 | 14 | 19 | 4.098 | 1.742-9.615 | 0.001* |
| Surgical duration (min) | | | | | |
| ≤90 | 4 | 18 | 1.000 | | |
| >90 | 28 | 101 | 0.802 | 0.251-2.561 | 0.709 |
| Body mass index (kg/m²) | | | | | |
| <18.5 | 1 | 4 | 3.226 | 0.033-2.930 | 0.635 |
| 18.5~24.0 | 3 | 78 | 1.000 | | |
| >24.0 | 28 | 37 | 32.258 | 7.194-142.857 | 0.002* |
| Tobacco use | | | | | |
| Yes | 22 | 67 | 1.000 | | |
| No | 10 | 52 | 0.631 | 0.283-1.406 | 0.260 |
| Surgical removal of internal fixation | | | | | |
| Yes | 10 | 21 | 0.471 | 0.195-1.141 | 0.095 |
| No | 22 | 98 | 1.000 | | |
| Intraoperative blood loss (mL) | | | | | |
| ≤200 | 6 | 45 | 1.000 | | |
| >200 | 26 | 74 | 2.639 | 1.007-6.897 | 0.048* |

OR: Odds ratio; CI: Confidence interval; * Signifies statistical significance (p<0.05); Stiffness group refers to the patients with secondary Shoulder stiffness after open reduction and internal fixation (ORIF) of proximal humerus fracture, while non stiffness group refers to the patients with no secondary Shoulder stiffness after ORIF of proximal humerus fracture.

**TABLE II**
Multifactor analysis results of influencing factors of shoulder stiffness after ORIF of proximal humeral fractures

| Risk factors | Partial regression coefficient | SE | Wald $\chi^2$ | aOR | 95% CI | *p* |
|---|---|---|---|---|---|---|
| High-energy injury | 1.549 | 0.508 | 9.294 | 7.706 | 3.564~15.579 | <0.001* |
| Time from injury to surgery >1 week | 1.264 | 0.754 | 5.645 | 5.275 | 1.7321~9.472 | 0.031* |
| Body mass index (>24.0 kg/m$^2$) | 1.475 | 0.497 | 4.263 | 4.427 | 1.671~11.722 | 0.023* |
| Intraoperative blood loss >200 mL | 1.832 | 0.933 | 2.472 | 3.565 | 0.326~31.935 | 0.372 |

SE: Standard error; aOR: Adjusted odds ratio; CI: Confidence interval; Wald $\chi^2$, Chi-square test; * Signifies statistical significance (p<0.05).

operation duration, intraoperative blood loss, surgical removal of internal fixation, and dominant hand) were compared between groups with the chi-square test. First, a multivariate unconditional logistic regression model analysis was conducted to validate the factors with statistically significant differences in single factor analysis, and the correlation strength was determined using ratio analysis [adjusted odds ratio (aOR)] and 95% confidence interval (CI). A *p*-value <0.05 was considered statistically significant.

## RESULTS

The mean follow-up duration was 31.8±12.6 (range, 12 to 68) months. Eighty-one cases were on the dominant side, and 70 cases were on the nondominant side of the affected limb. According to the Neer classification system, the fracture type was type 3 in 112 patients and type 4 in 39 patients. According to the AO classification system, the fracture type was B1.1 in 19 patients, B1.2 in 92 patients, C1 in 13 patients, and C3 in 27 patients. The mechanism of injury was high-energy in 58 patients, vehicle-related in 28, falling-related in 18, and sports-related in 12. Ninety-three patients had low-energy injuries, such as simple falls or falls from standing height. Fourteen patients were complicated with other fractures: one with an ipsilateral radial head fracture, one with an ipsilateral distal radius fracture, three with a pelvic fracture, two with an ipsilateral tibial plateau fracture, four with an ipsilateral or contralateral multiple rib fracture, two with an ipsilateral femoral supracondylar fracture, and one with an ipsilateral ankle fracture. None of the patients experienced any complications, such as nonunion, malunion, infection, loose internal fixation, or breakage of steel plates or screws. At the last follow-up, all fractures had healed. Thirty patients underwent complete removal of internal fixation.

The patients in the stiffness group were followed up for 30.7±9.5 months, and the non-stiffness group was followed up for 32.4±8.9 months. Single-factor analysis showed that there were statistically significant differences between the two groups in terms of the factors of injury energy (p<0.001), time from injury to surgery (p=0.001), BMI (p=0.002), and intraoperative blood loss volume (p=0.048, Table I). Among them, high-energy injuries, time from injury to surgery >1 week, BMI >24.0 kg/m$^2$, and intraoperative blood loss were risk factors that could lead to postoperative shoulder stiffness. However, other factors, such as age, sex, tobacco use, diseased side, surgical duration, surgical removal of internal fixation, Neer classification of fractures, AO classification of fractures, existence of additional fractures, and dominant hand, did not have statistically significant differences (p>0.05, Table I). In the stiffness group, the score of ASES (25.33±5.86) and UCLA (13.56±2.42) were lower than the ASES (97.15±3.49) and UCLA (34.07±1.11) scores in the non-stiffness group (p<0.0001).

Multivariate unconditional logistic analysis showed that high-energy injuries (aOR=7.706, 95% CI: 3.564-15.579, p<0.001), time from injury to surgery >1 week (aOR=5.275, 95% CI: 1.7321-9.472, p=0.031) and BMI >24.0 kg/m$^2$ (compared to BMI between 18.5 and 24.0 kg/m$^2$; aOR=4.427, 95% CI: 1.671-11.722, p=0.023) were independent risk factors for shoulder stiffness after proximal humeral fracture surgery. Intraoperative blood loss was not an independent risk factor for shoulder stiffness after ORIF of proximal humeral fractures (Table II).

## DISCUSSION

For the treatment of proximal humeral fractures, short-term fixation is a widely recognized nonsurgical treatment option that has shown good clinical results in patients with stable and partially displaced fractures.[12,13] However, the proportion of patients with displaced fractures of the proximal humerus has increased by 50% over the past 30 years.[14] Among various surgical techniques, locking plates are the most widely used to treat complex proximal humeral fractures.[15] In a study on rotator cuff repair, it was found that out of 489 patients, 24 (4.9%) experienced limited shoulder movement after surgery, and patients who were unable to perform shoulder movement due to shoulder discomfort were defined as shoulder stiffness patients.[16] However, this definition is only based on the patients' own feelings and is subjective. Chung et al.[17] stated that the criteria for shoulder stiffness were forward flexion <120°, external rotation of the arm at the body side <30°, or internal rotation of the back below the L3 level. Since these three parameters are easily obtained in the outpatient clinic, we used forward shoulder flexion <120°, external arm rotation <30° at the side of the body, and internal back rotation below the L3 level as the diagnostic criteria for the classification of patients.

Low-energy injuries, such as falling from a standing height, account for approximately 87% of the injury mechanisms of proximal humeral fractures.[18] The rest are mostly caused by high-energy injuries, such as falls, vehicle accidents, and other high-energy mechanisms.[19] High-energy injuries are usually associated with soft tissue injuries, comminuted fractures, craniocerebral trauma, and multiple fractures, which may have adverse effects on joint function recovery and overall clinical prognosis.[20] The present study found that high-energy injuries were an independent risk factor for postoperative shoulder stiffness (aOR=7.706, 95% CI: 3.564-15.579, p<0.001). However, whether high-energy injuries are an independent risk factor for traumatic shoulder stiffness has not been reported. Therefore, more prospective clinical studies with a large sample size are needed to further confirm these findings. We suggest that for patients with proximal humeral fractures caused by high-energy injuries, a complete preoperative protocol and a postoperative functional recovery plan should be developed.

For elderly patients with many medical complications, a comprehensive evaluation should be performed before surgery or conservative treatment should be selected after injury. However, if fracture end displacement occurs during follow-up, patients do not seek medical treatment immediately after injury, the time from injury to surgery is relatively long, or the shoulder needs a long immobilization time, an adverse impact on the recovery of shoulder function is likely. This study found that patients who underwent surgery more than one week after injury were at five times higher odds of having shoulder stiffness (95% CI: 1.7321~9.472, p=0.03). Therefore, on the basis of actively improving preoperative examination and comprehensive evaluation of the patient's condition, the time from injury to operation should be controlled within one week as much as possible to reduce the risk of postoperative shoulder stiffness. In addition, a green channel for auxiliary examination and consultation can be established, the preoperative evaluation time can be shortened, and the fracture can be firmly fixed as soon as possible.

Treatment of obese patients is challenging because increased adipose tissue, increased anatomical depth, and a number of comorbidities can complicate surgery. A study found a positive correlation between an increase in BMI and an increased risk of postoperative complications in elbow, forearm, and hand surgery.[21] Kashanchi et al.[22] identified obesity as an independent risk factor for complications after arthroscopic rotator cuff repair surgery (OR=1.72, p=0.005). Similar to previous studies, this study also found BMI >24.0 kg/m$^2$ as an independent risk factor for shoulder stiffness (compared to BMI between 18.5 and 24.0 kg/m$^2$, aOR=4.427, 95% CI: 1.671-11.722, p=0.023). Therefore, we believe that weight control combined with postoperative rehabilitation exercises for patients with proximal humeral fractures can significantly promote the functional recovery of the shoulder. The World Health Organization BMI cutoff value is internationally used (BMI between 25.0 and 29.9, overweight; BMI ≥30, obesity).[23] The Working Group on Obesity in China proposed a BMI >24.0 kg/m$^2$ as the overweight cutoff point for Chinese adults.[24] This study shows that it is beneficial to control BMI to be below 24.0 kg/m$^2$ to reduce the incidence of shoulder stiffness. In the current study, the body weight of the patients was classified according to BMI, which was used as the criterion for judging whether the patients were obese. Compared to directly measuring body fat, BMI may underestimate the prevalence of obesity since it does not take into account the patient's body water, muscle mass, or bone density.[25]

Intraoperative blood loss >200 mL was more prominent in the stiffness group compared to the nonstiffness group (p=0.048), but it was not an independent risk factor for shoulder stiffness (aOR=3.565, 95% CI: 0.326-31.935, p=0.372). Therefore, more prospective clinical studies with a large sample size are needed for further exploration. In this study, the proportion of patients with a history of smoking in the stiffness group and the non-stiffness group was compared. The incidence of postoperative shoulder stiffness was not increased in patients with a history of smoking ($p=0.260$). This may be related to the inclusion of more patients with no smoking history in the stiffness group, leading to a bias in the results. Nicotine, tar, and carbon monoxide in cigarettes, and these toxic substances can shrink blood vessels, resulting in oxygen transport dysfunction.[26] Smoking can cause degeneration of the supraspinatus and infraspinatus tendons, which can affect the functional activities of the shoulder.[27] In a meta-analysis that included 13 items, including 16,172 patients, of whom 6,081 were smokers, a correlation was found between smoking and shoulder dysfunction and symptoms in patients.[28] Smokers reported higher shoulder pain and poorer shoulder function than nonsmokers. Therefore, when patients with proximal humeral fractures undergo functional rehabilitation after ORIF, effective tobacco control intervention and fully informing patients about the hazards of smoking should be important measures to prevent shoulder stiffness. In a study of 212 patients who underwent ORIF for the treatment of a two-part or three-part fracture of the proximal humerus, statistical significance was found between the duration of surgery and the finding of postoperative shoulder stiffness ($p<0.05$).[29] However, the mechanism is still unclear. The current hypothesis of its role in shoulder stiffness is that prolonged intraoperative contraction of the attached muscles on the humerus, such as the deltoid muscle or rotator cuff, may lead to muscle fatigue or weakness or that prolonged surgery may cause peripheral nerve traction and compression, which may lead to limited shoulder mobility after surgery. However, this factor ($p=0.709$) was not statistically significant in the results of this study, which may be related to the relatively single subject of the study or the insufficient number of patients.[29] The ASES and UCLA scores of the stiffness group were lower than those of the non-stiffness group, but there was no clear guiding significance for the risk factors, which may be related to the relatively insufficient number of patients.

This study had several limitations. First, this was a retrospective study, and all the data were collected retrospectively from the medical records of the patients, which may be biased from the actual data. Second, the sample size was relatively small, so the number of risk factors studied was limited. Hence, a prospective study with a larger sample size and a randomized controlled trial should be conducted to identify the risk factors for shoulder stiffness. In addition, the Charlson Comorbidity Index, the number of comorbidities, the occupation of patients, socioeconomic status, the postoperative rehabilitation exercise program, and level of patient compliance will have a greater impact on the recovery of shoulder function, but this study did not explore such relevant issues.

In conclusion, high-energy injuries, time from injury to surgery >1 week, and BMI >24.0 $kg/m^2$ were identified as independent risk factors for shoulder stiffness after proximal humeral fracture surgery, which significantly affect the quality of life of patients after surgery. Therefore, enough attention should be paid to shorten the preoperative waiting time as much as possible, actively educate patients before surgery, manage weight, and increase compliance, which will ultimately improve patient satisfaction. For fractures caused by high-energy injuries, patients and their families should be fully informed of the possibility of postoperative shoulder stiffness, and a reasonable surgical plan should be formulated to shorten the operation time and reduce intraoperative blood loss as much as possible.

**Ethics Committee Approval:** The study protocol was approved by the Baotou No.3 Hospital Ethics Committee (date: 17.11.2021, No. 04). The study was conducted in accordance with the principles of the Declaration of Helsinki.

**Patient Consent for Publication:** A written informed consent was obtained from each patient.

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions:** Conceptualization and writing-original draft preparation: L.W.; Methodology: F.L.; Software and data curation: J.R.; Resources: H.S.; Writing-review and editing: B.L., J.L.; Supervision: B.L.; Contributed equally to this work and share first authorship: L.W., F.L., J.R.; All authors have read and agreed to the published version of the manuscript.

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