Original Article

Effects of supplementation with a combination of β-hydroxy-β-methyl butyrate, L-arginine, and L-glutamine on postoperative recovery of quadriceps muscle strength after total knee arthroplasty

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Objectives: Total knee arthroplasty (TKA) performed in knee osteoarthritis patients is reported to be immediately followed by a decrease in quadriceps muscle strength. We investigated the effects of supplementation with a combination β-hydroxy-β-methyl butyrate, L-arginine, and L-glutamine (HMB/Arg/Gln) on the postoperative recovery of quadriceps muscle strength in patients after TKA. Methods: Study subjects were 23 patients (12 women; mean age: 70.5) who underwent TKA. The patients were randomly allocated into the control group or the group that consumed HMB/Arg/Gln supplementation (HMB/Arg/Gln group). HMB/Arg/Gln supplementation or control food were consumed for 5 days before the surgery and for 28 days after the surgery, and maximal quadriceps strength was measured at 7 days before the surgery, and at 14, 28 and 42 days after the surgery. During the study, total energy expenditure was measured using a lifestyle recording device. The two groups followed the rehabilitation in the same way. Results: The maximal quadriceps strength was 1.1±0.62 Nm/Kg before surgery and 0.7±0.9 Nm/Kg after surgery 14 days in the control group (p=0.02), and 1.1±0.3 Nm/Kg before surgery and 0.9±0.4 Nm/Kg after surgery 14 days in the HMB/Arg/Gln group. Although the control group experienced a significant loss of muscle strength after the surgery, the HMB/Arg/Gln group did not. There was no significant difference in total energy expenditure between the two groups. Conclusions: Consuming HMB/Arg/Gln supplementation may suppress the loss of muscle strength after TKA. Intervention with exercise and nutrition appears to enable patients to maintain their quadriceps strength.

Key Words: β-hydroxy-β-methyl butyrate (HMB), nutritional food, quadriceps muscle strength, total knee arthroplasty (TKA), knee osteoarthritis

INTRODUCTION

End-stage knee joint failures caused by osteoarthritis or rheumatoid arthritis are subject to the total knee arthroplasty (TKA). Patients receiving TKA have been increasing in number every year with the aging population increase.1-3 It is reported however that TKA generates a loss of quadriceps strength4-5 and that the knee osteoarthritis case tends to generate greater loss in leg muscle mass and strength than the rheumatoid arthritis.6 Decreased leg muscle strength can lead to falling and fractures, and is a key determinant factor for independency in the elderly; knee extension strength is regarded as particularly important for walking ability.7 A study on patients after surgery for femoral neck fracture reported that walking ability is affected by leg muscle strength,8 and in recent situations in which hospital stays are kept to a minimum, leg muscle strength is presumed also to be a major factor for improving treatment outcomes and patient quality of life (QOL) after TKA.9 Factors that can cause a loss of muscle strength after TKA include muscle damage caused by the invasive surgery, and disuse muscular atrophy caused by the inactive lifestyle after surgery. Measures against these risk factors include the development of less invasive surgical methods, and more effective approaches to early ambulatory mobilization after surgery.

Exercise and nutrition have been attracting attention as factors affecting skeletal muscle mass in elderly patients with sarcopenia, and considerable research has verified that incremental-load resistance training and protein (amino acid) supplementation are effective in increasing muscle mass and strength.10,11 Therefore, the combination of amino acid (HMB, Arg, and Gln) supplementation in elderly patients is expected to increase quadriceps muscle strength and is expected to improve the quality of life (QOL) after TKA.

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muscle mass and muscle strength.10–13 In recent years, some reports have indicated that consuming β-hydroxy-β-methyl butyrate (HMB) supplementation, which is a metabolite of leucine, promotes an increase in lean body mass and muscle strength as a result of resistance training.13,14

Previous studies have also reported the effects of HMB supplementation intake during resistance training in healthy adults.13,16 As well as reported that when HMB is consumed during bed rest, the reduction in muscle mass is slowed.17 It has also been suggested that HMB has anti-catabolic properties. Several experimental studies with HMB have demonstrated to attenuate protein degradation and induction of the ubiquitin-proteasome proteolytic pathway during catabolic states.18,19 In human studies, HMB has been shown to attenuate protein muscle degradation in young adults during resistance training and to prevent loss of lean body mass during 10 days of bed rest in older adults.13–17 Glutamine is a conditionally essential amino acid under severe stressful conditions.20 Glutamine administration has been reported to improve nitrogen balance and length of stay in patients following surgery as well as decrease the rate of infection in blunt trauma patients.21 Arginine is also a conditionally essential amino acid under severe stressful conditions. Animal studies have shown that arginine improved protein anabolism in rats following burns as well as trauma.22 Arginine administration improved wound healing and protein anabolism in healthy elderly.23

METHODS

Subjects and study design

We screened 139 patients who were diagnosed as having knee osteoarthritis and scheduled for TKA at our hospital between August 2012 and July 2013. Of these patients, 23 knee osteoarthritis patients (11 men and 12 women) between 65–80 years of age (mean age 70.5±5.4), who were capable of walking, and gave their own consent to participate in this study, were enrolled as study subjects. Patients with poorly controlled diabetes, hypertension, heart disease, liver disease, or kidney disease, and patients using or scheduled to use drugs or some supplements that may have an effect on muscle strength recovery or muscle mass, were excluded from the study.

This study was conducted with the approval of the institutional review board of Kugawa Hospital for Orthopaedic Surgery (Approval Number 2012-1).

Study design

This was a randomized controlled study. Figure 1 shows a flow chart of the study. Study subjects were randomly divided into two groups; 10 patients were given orange juice (control group) and 13 patients were given the HMB/Arg/Gln supplementation dissolved in 240–300 mL of water (HMB/Arg/Gln group). The orange juice contained 226 kcal of energy and 280 mg of protein (daily dose), and the HMB/Arg/Gln supplementation contained 2,400 mg of HMB, 14,000 mg of L-glutamine, 14,000 mg of L-arginine, 158 kcal of energy, and 0 mg of protein (daily dose). Both groups took their beverage for the 5 days before the surgery and for the 28 days from the day after surgery daily twice after meals.

Postoperative rehabilitation

Rehabilitation was conducted in accordance with our hospital’s protocol for TKA postoperative patients. Strength training and range-of-motion exercises started in bed from the first postoperative day, and the walking training (full load) started on the second postoperative day. A wheelchair was not used after the start of walking

![Flowchart of the study](image-url)

**Figure 1.** A flowchart of the study
training, and the patient moved using a walker. Rehabilitation was performed 5 days a week during the hospital stay. The patient participation in rehabilitation after discharge was voluntary, but all patients received home exercise guidance at the time of discharge.

**Muscle strength testing**

BIODEX (BDX-4Q, Biodex, USA) was used to measure both the operated-side knee extension strength and non-operated-side knee extension strength, before surgery and on postoperative days 14, 28, and 42. The isometric contraction tests were conducted 5 times on each day, five-second intervals. Participants were given, verbal instruction and practiced the procedure twice. And we took the average among 5 tests cutting the maximum result and the minimum.

**Measurement of the total amount of exercise**

The lifestyle recorder, Life coder GS (Suzuken, Japan), fitted with an acceleration sensor was used to record the physical activity during the 5 days before the surgery and from post operatively from days 2 to 42. From the measured data, the 1-day mean total energy expenditure during specific periods of time, namely, before the surgery (5 days before the surgery to the day before the surgery), postoperative day 2 to day 14, postoperative day 15 to day 28, and postoperative day 29 to day 42, were calculated.

**Computerized tomography (CT) scans**

The mid-thigh on both the operated side and the non-operated side was imaged using CT at the start and at the end (postoperative day 42) of the study. The rectus femoris cross-sectional area was measured using the image processing software Image J (National Institutes of Health, USA).

**Body weight**

Each patient’s body weight was measured before the surgery, and on postoperative days of 14th, 28th, and 42nd.

**Dietary intake**

During the hospital stay, patients were fasted on the day of surgery, and provided with hospital food from postoperative day 1. For 3 days after the surgery, dietary intakes were recorded, until the patient could eat all of the provided food. The orange juice and the dietary HMB/Arg/Gln supplementation intake volumes were recorded using a report form. Patients were prohibited from consuming glutamine and arginine preparations for the duration of the study.

**Statistical analyses**

The patient backgrounds at the start of the study, their ages, weights, knee extension strengths, rectus femoris cross-sectional areas, and total energy expenditure were compared between the control group and the HMB/Arg/Gln group using the unpaired t-test. We used two-way repeated measurement ANOVA with muscle strength (pre/postoperative) and supplementation (orange juice/ (HMB/Arg/Glu)). The relative change ratio of total energy expenditure at each measurement to that at the start were also compared within groups using the paired t-test.

In addition, the changes in total energy expenditure and rectus femoris cross-sectional area at each measurement were calculated from the values at the start of the study, and compared between the control group and HMB/Arg/Gln group using the unpaired t-test. All numbers are indicated as mean±SD. A p-value <0.05 was considered to indicate a statistically significant difference between groups. Calculations were performed using statistical software (IBM SPSS Ver.12).

**RESULTS**

All 23 participants completed the entire process without dropping out from the study. Table 1 shows the result of body weight, operated-side and non-operated-side of rectus femoris muscle cross-sectional area (cm$^2$), and total energy expenditure from all subjects during the study. Figure 2 and 3 show the knee extension muscle strength of all subject during the study.

**Rehabilitation and hospital stay**

There were no significant differences in body weight, knee extension strength, muscle cross-sectional area or total energy expenditure between the control group and the HMB/Arg/Gln group (Table 1). Rehabilitation was performed in accordance with the hospital’s protocol for TKA postoperative patients (muscle strength training and range-of-motion exercises in bed from postoperative day 1, and ambulation from postoperative day 2). The rehabilitation period was 12.8±3.2 days for the control group, and 13.1±2.4 days for the HMB/Arg/Gln group (p=0.54). The length of hospital stay was 19.1±3.7 days for the control group, and 18.9±3.3 days for the HMB/Arg/Gln group, and there was no significant difference between the two groups (p=0.85).

**Dietary intake**

Calculating intake of orange juice or HMB/Arg/Gln supplementation when all-quantity is based at 100%, the amounts of orange juice and HMB/Arg/Gln consumed were 98±2.3% for the control group and 99±3.3% for the HMB/Arg/Gln group, respectively. During the hospital stay, patients were provided with hospital food from postoperative day 1. The 1-day mean total energy intake was 1,737±104 kcal for the control group and 1,765±107 kcal for the HMB/Arg/Gln group, of which protein accounted for 62.6±4.2 g in the control group and 63.7±4.1 g in the HMB/Arg/Gln group. The amount of dietary intake was assessed in compliance with our hospital’s protocol. At our hospital, the amount of food for the 3 days from the day after surgery is assessed, and only the patients who could not eat all of the provided food during that time were checked thereafter. All of the study participants consumed almost all of their food for the first three days after the surgery (from the day after surgery to postoperative day 3).

**Body weight**

The body weight changes showed in Figure 2. The mean body weight of the control group was 64.7±9.7 kg at the start and 64.9±9.3 kg at the end of the study, and there was no significant difference between the two groups.
Table 1. Patients' demographics, rectus femoris cross-sectional area and total energy expenditure

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=10)</th>
<th>HMB/Arg/Gln group (n=13)</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>14 days</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.8</td>
<td>NA</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>64.7±9.7</td>
<td>63.5±9.5</td>
</tr>
<tr>
<td>Operated-side muscle cross-sectional area (cm²)</td>
<td>542±164</td>
<td>NA</td>
</tr>
<tr>
<td>Non-operated-side muscle cross-sectional area (cm²)</td>
<td>577±140</td>
<td>NA</td>
</tr>
<tr>
<td>Total energy expenditure (kcal/day)</td>
<td>1416±156</td>
<td>1506±143</td>
</tr>
</tbody>
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NA: not available.
The mean body weight of the HMB/Arg/Gln group was 63.6±10.7 kg at the start and 62.7±10.2 kg at the end. Although the difference was not significant, there was a tendency toward weight loss (p=0.06).

**Muscle strength**

In the control group, the mean operated-side knee extension strengths were 1.1±0.62 Nm/Kg preoperatively, 0.7±0.9 Nm/Kg on postoperative day 14, 0.9±0.9 Nm/Kg on postoperative day 28, and 0.9±0.6 Nm/Kg on postoperative day 42. In the HMB/Arg/Gln group, they were 1.1±0.3 Nm/Kg preoperatively, 0.9±0.4 Nm/Kg on postoperative day 14, 1.0±0.4 Nm/Kg on postoperative day 28, and 1.0±0.4 Nm/Kg on postoperative day 42. In a comparison of preoperative and postoperative muscle strengths, the control group demonstrated a significant loss of muscle strength postoperatively between before surgery and postoperative day 14 (p=0.02). In the HMB/Arg/Gln group, there was no significant difference between the preoperative muscle strengths and each of the postoperative values (Figure 2).

The non-operated-side knee extension strengths of the control group were 1.3±0.7 Nm/Kg preoperatively, 1.1±0.6 Nm/Kg on postoperative day 14, 1.3±0.6 Nm/Kg on postoperative day 28, and 1.2±0.5 Nm/Kg on postoperative day 42. In the HMB/Arg/Gln group, they were 1.3±0.4 Nm/Kg preoperatively, 1.3±0.3 Nm/Kg on postoperative day 14, 1.3±0.4 Nm/Kg on postoperative day 28, and 1.2±0.3 Nm/Kg on postoperative day 42. Comparison of the preoperative and postoperative muscle strengths showed that there was no significant difference between the preoperative muscle strength and any of the postoperative values, in both the control group and the HMB/Arg/Gln group (Figure 3).

**Figure 2.** Changes in the operated-side knee extension strengths. The operated-side knee extension strengths were measured before surgery and on postoperative days 14, 28, and 42. We used two-way repeated measurement ANOVA with muscle strength (pre/postoperative) and supplementation (orange juice/ (HMB/Arg/Glu)) (**p=0.02**)

**Figure 3.** Changes in the non-operated-side knee extension strengths. The non-operated-side knee extension strengths were measured before surgery and on postoperative days 14, 28, and 42. The knee extension strength before surgery and at each measurement after surgery was compared within groups using the paired t-test.
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Amount of exercise
The change in 1-day mean total energy expenditure at each period after surgery relative to the preoperative total energy expenditure was calculated. Neither an intra-group comparison between before surgery and each period after surgery, nor an inter-group comparison between the control group and the HMB/Arg/Gln group at each assessment period showed a significant difference.

In the control group, the change was 6.3±3.1% postoperative 13 days 2nd to 14th, 2.2±3.7% over 14 days from15th to 28th, and 6.3±4.9% over 14 days from 29th to 42nd. In the HMB/Arg/Gln group, the change was 2.2±6.7% over the postoperative 13 days from 2nd to 14th, 4.8±6.0% over 14 days from 15th to 28th, and 2.1±6.0% over 14 days from 29th to 42nd (Table 1).

Rectus femoris cross-sectional area
Changes in the rectus femoris cross-sectional area before the surgery and 42 days after the surgery were calculated. With respect to the preoperative cross-sectional area was -4.3±26.2% in the control group and 10.0±20.5% in the HMB/Arg/Gln group, where the HMB/Arg/Gln group showed an increasing trend (p=0.15). The change in the rectus femoris cross-sectional area of the non-operated side was -3.6±33.8% in the control group and 9.1±16.6% in the HMB/Arg/Gln group. There was no significant difference between the groups (Table 1).

DISCUSSION
Although TKA applied to knee osteoarthritis improves walking ability through pain relief, the surgery is reported to generate a loss of quadriceps strength.4,5 Various measures to deal with problem have been examined, such as devising better surgical methods and starting some standing ambulation after surgery. However, for elderly subjects, the progression of sarcopenia, in which skeletal muscle mass and muscle strength decrease, causes a reduction of physical ability and independence in daily living. In the elderly, the mechanism of the skeletal muscle mass reduction is not fully understood. For the improvements of aging, chronic diseases, disuse of skeletal muscle, and unfavourable nutritional states are thought to be involved, and there are some reports that complex intervention with exercise and nutrition were reported to be effective.6-13 Patients with knee osteoarthritis lose their quadriceps muscle strength, which is a determinant factor of walking ability, because knee osteoarthritis is a chronic musculoskeletal disease associated with aging, and the pain caused by the disease restricts activities of daily living.24 In the TKA perioperative period, the invasiveness of the surgery causes a loss of quadriceps muscle strength. Therefore, we hypothesized that introducing a nutritional approach using HMB/Arg/Gln supplementation (which promotes protein synthesis) to postoperative patients whose demand for protein is increased by the invasiveness of the surgery, could possibly enhance the recovery of quadriceps strength, and we investigated this hypothesis with patients in the TKA perioperative period.

We investigated the effects of consuming HMB/Arg/Gln supplementation on quadriceps strength and mass in 23 patients who underwent TKA because of knee osteoarthritis, and found that the control group had significant muscle strength loss after surgery, which is consistent with previous reports,4,5 whereas the HMB/Arg/Gln group did not show significant muscle strength loss. The rectus femoris cross-sectional area of the HMB/Arg/Gln group had a greater rate of increase than that of the control group, although the difference was not statistically significant. In addition, the total amount of exercise, which affects muscle strength and mass, was not significantly different between the control group and the HMB/Arg/Gln group at all time periods. Some previous studies reported that the consumption of HMB/Arg/Gln supplementation results in the preservation of muscle strength and mass,13-17 and consistently, we showed here that in the TKA perioperative period as well, the consumption of HMB/Arg/Gln supplementation can suppress the loss of muscle strength.

In this study, we focused on HMB, which has been reported to promote protein synthesis25 and suppress protein breakdown in the body.14,26 The proposed mechanisms of HMB include stimulated protein synthesis via activation of mammalian target of rapamycin (mTOR) pathway27 and attenuation of protein degradation via, firstly, inhibition of ubiquitin proteasome pathway19 as well as, secondly, inhibition of proapoptotic pathways in catabolic states.28 L-arginine is a precursor for muscle protein synthesis as well as for nitric oxide, which is thought to increase muscle blood flow and thereby has been proposed to stimulate muscle protein synthesis.29 Glutamine may promote protein synthesis, and inhibit protein degradation.30 Glutamine is especially important during times of severe stress caused by surgery or illness. It has been reported that glutamine levels within skeletal muscles decrease following surgery.30,31,32 This decrease is accompanied by a decrease in muscle protein synthesis.13,15 Glutamine administration is reported to help correct the decreased glutamine level and enhance net protein synthesis in patients undergoing elective surgery.31

In patients receiving glutamine after surgery, protein synthesis is increased, leading to a quicker recovery. We hypothesized that when patients, who are in a state of elevated demand for protein because of invasive surgery, consume HMB/Arg/Gln supplementation, the suppression of protein breakdown and the promotion of protein synthesis in the skeletal muscle are induced, leading to an increase in muscle mass and maintenance of muscle strength. We also believe that the control group might have a loss of muscle strength because the invasiveness of the surgery increases protein breakdown. Factors that affect skeletal muscle strength and mass include exercise and nutrition, and our results suggest that the consumption of nutritional food on its own can increase muscle strength, because there was no difference in the amount of exercise between the 2 groups in this study.

There is no prior research to our knowledge showing that HMB/Arg/Gln supplementation is effective in the perioperative period of orthopaedic surgery. In studies performed on healthy subjects, consuming branched-chain amino acids alone did not increase muscle strength,32 and many studies in which muscle growth and strengthening were observed with training alone involved a considerable amount of high-intensity training.10,33 As a reason for this, the muscle damage caused by training
promotes the synthesis and breakdown of skeletal muscle protein, and having a higher concentration of amino acids in the blood due to amino acid consumption causes more protein synthesis than protein breakdown, which results in the accumulation of skeletal muscle protein and the growth of muscle.34

However, in clinical practice, a training regimen of sufficiently high intensity to affect muscle strength or mass is often difficult after TKA, because of the pain caused by the invasive surgery. There are also some patients with reduced motivation and decreased amount of daily living activities because of the pain. Due to the invasiveness nature of TKA surgery, there is an elevated demand for protein. Our nutritional approach, such as HMB/Arg/Gln supplementation, to the perioperative TKA management, will make it possible to maintain muscle strength without affecting the patient’s motivation and activities.

This approach may also resolve the problems that arise after TKA. Decreased leg muscle strength is a factor for falling and fractures, and is a major factor determining whether elderly patients can independently perform daily living functions, and knee extension strength is particularly important for walking.3 A previous report also indicated that patients who undergo TKA have a 20% to 30% reduction in muscle strength and walking ability, even at 1 year after the surgery, compared with same age controls.3 Muscle strength and daily living activity level are closely related. Preventing as much as possible the loss of quadriceps strength that occurs after surgery has the potential to lead to improved walking ability and daily living activity level, and may contribute to the prevention of falls and improvement of QOL.

The following are limitations of this study. This study is a pilot study performed with a small number of subjects, and therefore, verification of these results with an increased number of subjects is required. In addition, conforming to our hospital’s postoperative TKA protocol, the amount of food consumption was assessed only for the 3 days after surgery. However, investigating the effects of HMB/Arg/Gln supplementation in more detail would require assessing dietary intake during the entire study period. In the future, a large-scale, double-blind study should be performed, and whether a similar effect is also obtained under conditions in which the postoperative protocol is different should be verified in a multicentre study.

**Conclusions**

We investigated the effects of consuming HMB/Arg/Gln supplementation on postoperative recovery of quadriceps muscle mass and strength in patients who underwent TKA because of knee osteoarthritis. We found that consuming HMB/Arg/Gln supplementation could suppress the loss of quadriceps muscle strength that occurs after TKA. Patients with knee osteoarthritis are reported to experience loss of quadriceps muscle strength from before surgery, and have even more muscle strength loss after the surgery than same age controls. Quadriceps muscle strength is a determinant factor for walking ability and an important factor for falling risk, and it is expected to have a major effect on QOL. Complex intervention with exercise and nutrition is considered effective for improving muscle strength in the elderly with sarcopenia.

It is expected that performing a similar intervention in the perioperative period of orthopaedic surgery for the elderly could lead to fall prevention and early improvement in physical function, and could contribute to promoting a healthy life.

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**AUTHOR DISCLOSURES**

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联合补充 β 羟基 β - 甲基丁酸、L-精氨酸和 L-谷氨酰胺对全膝关节置换术后股四头肌肌肉力量恢复的效果

目的：有报道称膝关节炎病人全膝关节置换术后，紧接着就是股四头肌肌肉力量的减小。我们研究了联合补充 β 羟基 β - 甲基丁酸、L-精氨酸和 L-谷氨酰胺（HMB/Arg/Gln）对全膝关节置换患者术后股四头肌肌肉力量恢复的效果。

方法：研究对象为进行全膝关节置换的 23 位患者（平均年龄为 70.5 岁，其中 12 名为女性）。患者被随机分配进对照组和 HMB/Arg/Gln 补充组。手术前补充 5 天和手术后补充 28 天的 HMB/Arg/Gln 或对照食物。分别在术前 7 天，术后 14 天、28 天和 42 天测定最大股四头肌力量。在研究过程中，使用一个生活方式记录装置测定总的能量消耗。随后两组以同样的方式康复。

结果：对照组手术前最大股四头肌力量为 1.1±0.62 Nm/Kg，手术后 14 天为 0.7±0.9 Nm/Kg （p=0.02）；HMB/Arg/Gln 组手术前最大股四头肌力量为 1.1±0.3 Nm/Kg，手术后 14 天为 0.9±0.4 Nm/Kg。对照组手术后肌肉力量损失显著，但 HMB/Arg/Gln 组没有。两组之间能量消耗没有显著差异。

结论：补充 HMB/Arg/Gln 可以抑制全膝关节置换术后肌肉力量的损失。运动和营养干预似乎能够让患者保持他们的股四头肌力量。

关键词：β 羟基 β - 甲基丁酸（HMB）、营养食物、股四头肌肌力、全膝关节置换术（TKA）、膝关节骨性关节炎