Abstract. The effect of protein intake on rat pressure ulcer healing was evaluated. One hundred rats were numbered according to body weight and then they were randomly divided into 4 groups (n=25) using the random number table. After rat models of stage II pressure ulcer were established, they were fed with feed containing different protein levels (10, 15, 20 and 25%). Healing time, pressure ulcer area, body weight, albumin (ALB) and hemoglobin (Hb) levels among groups were compared. Hematoxylin and eosin (H&E) staining was also performed to observe pressure ulcer tissue structure. In the healing process of pressure ulcer, rats with 20% protein intake had the shortest healing time and the smallest pressure ulcer area. Body weight, ALB and Hb levels were much closer to the normal level. H&E staining result also suggested that the pressure ulcer healing degree of rats with 20% protein intake was much better than the others. Adequate protein intake is therefore conducive to pressure ulcer healing, while excessive or insufficient protein intake has negative impact on healing.

Introduction

The occurrence of pressure ulcer is a serious complication in health care centres (1). Reduced mobility, sensory impairment and poor nutritional status are the significant factors that increase the risk of pressure ulcer (2). Nutrition plays a key role in pressure ulcer healing and evidence has shown that nutritional support reduces the incidence of pressure ulcer in at-risk patients by 25% (3). Malnutrition is considered an important risk factor for the development of pressure ulcer (4). It has been proven that poor dietary intake is associated with the development of pressure ulcer as well as delayed healing time (5). Nutritional supplements are conducive to shortening the pressure ulcer healing time. In addition to regular food intake, providing an oral nutritional supplement is a logical way to promote tissue repair (6).

Prospective studies have shown that compared with patients whose nutritional intake was adequate, patients who had an adequate intake of protein had faster healing rates of pressure ulcers (7,8). Protein is essential for tissue cell repair and it is necessary for the repair of epithelial tissue (9). High protein diets may improve the healing of pressure ulcer. A number of studies have demonstrated that high protein intake is beneficial for pressure ulcer healing (8,10,11). In addition, many animal pressure ulcer models have been developed to test the effect of protein in this field (12,13). The aforementioned studies showed that high protein intake was helpful to pressure ulcer healing, but which protein level is most beneficial in the recovery from pressure ulcer remain to be determined. In this regard, the relevant research is rare.

In the present study, rat models of stage II pressure ulcer were established in order to investigate the effect of different level protein intake on healing of pressure ulcer.

Materials and methods

Animals. A total of 100 healthy male rats with a weight of 280±20 g were purchased from Experimental Animal Center of Hebei Medical University (Hebei, China), and the animal certificate number was 1607209. The use of animals was in accordance with the specification for laboratory animal use of People's Hospital of Hebei Province. The study was approved by the Ethics Committee of the Hebei General Hospital (Hebei, China).

Feed. Feeds with different protein intake were prepared (Table I). The protein levels of the feed were referred to Medical Laboratory Animal Standards promulgated by the Ministry of Health of the People's Republic of China in 1992. The standards stipulated that the protein level of the feed was 18-25%.

Grouping. The rats were housed at a constant room temperature (20-25°C) and humidity (40-60%) with standard feed (20% protein level) for one week. After being numbered according to body weight, the rats were randomly divided into 4 groups (groups A, B, C and D) using the random number table. The number of rats in each group was 25.
**Construction of rats with stage II pressure ulcer models.** All the rats were subjected to the same surgical procedure. Before surgery, they were fasted and water deprived for 8 h. Each rat was subjected to depilatory treatment with a skin area of 4x4 cm after they were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.3 ml/100 g body weight). The depilatory area was at the buttock on the left side of the back center line. After disinfection of the skin with 5% povidone-iodine, an incision with a length of 2 cm was performed. Fascia and tissue were separated by tweezers and a sterile steel disk (diameter 15 mm, thickness 0.3 mm, weight 0.6 g, autoclave sterilization) was implanted under the muscle. The incision was stitched with polyglycolic acid suture and 5% povidone-iodine was used to disinfect the suture to prevent infection. Three days after surgery, a magnetic disk (diameter 15 mm, thickness 2.5 mm, weight 2.6 g, with magnetic flux up to 1,500 gauss) was placed outside the skin in the area where the steel disk was placed (14). The pressure between the steel disk and magnet disk could cause pressure on the muscles and skin, which potentially led to local tissue ischemia. Two hours after pressurization, the magnets were removed for 0.5 h for ischemia reperfusion. This pressurization process was cycled 5 times a day and the stage II pressure ulcer was formed after 2-3 days of cyclic pressure operation. The criteria of successful stage II pressure ulcer models: The color of the pressure ulcer was dark red, the skin around the pressure ulcer was reddish, bleeding and exudate did not occur, obvious pain was showed by the rats.

**Intervention method.** Rats were fed with standard feed before and during the construction of stage II pressure ulcer models. The pressure ulcer area and body weight of the rats were measured and recorded immediately after the pressure ulcer was formed. After stage II pressure ulcer models were constructed, the rats in each group were fed with different protein levels of feed. The protein levels of the feed in groups A, B, C and D were 10, 15, 20 and 25%, respectively. The amount of feed was 20 g/rat/day and water was adequate.

**Monitoring of healing time, pressure ulcer area and rat body weight.** Healing time, pressure ulcer area, and rat body weight were monitored at different times. Evaluation criteria for pressure ulcer healing were as follows: The redness of the skin around the pressure ulcer had subsided, the scab of the pressure ulcer was shed, the new skin color was consisted with the surrounding normal skin color.

**Monitoring of ALB and Hb levels.** Before surgery, after surgery, and 3, 7 and 14 days after rat models were constructed, 3 rats in each group were randomly selected to obtain abdominal aortic blood samples. Albumin (ALB) and hemoglobin (Hb) levels were measured by spectrophotometry after the blood samples were diluted with Drabkin's solution and Bromocresol green, respectively.

**Hematoxylin and eosin (H&E) staining.** After the pressure ulcer models were established, pressure ulcer tissue samples were obtained. Pressure ulcer tissue samples of each group were obtained after 14 days feeding with different feeds. Each group was randomly selected to obtain abdominal aortic blood samples. Albumin (ALB) and hemoglobin (Hb) levels were measured by spectrophotometry after the blood samples were diluted with Drabkin's solution and Bromocresol green, respectively.

| Protein level | 10% | 15% | 20% | 25% |
|---------------|-----|-----|-----|-----|
| Corn flour (g) | 50  | 30  | 30  | 22  |
| Flour (g)     | 50  | 56  | 40  | 32  |
| Soy flour (g) | 3   | 4   | 8   | 15  |
| Soybean meal (g) | 4  | 4   | 10  | 8   |
| Bone meal (g) | 3   | 3   | 6   | 10  |
| Fish meal (g) | 6   | 3   | 6   | 10  |
| Salt (g)      | 0.05| 0.05| 0.05| 0.05|
| Cod liver oil (g) | 0.012| 0.012| 0.012| 0.012|
| Mineral (g)   | 0.16| 0.16| 0.16| 0.16|
| Multidimensional nutrients (g) | 0.12| 0.12| 0.12| 0.12|
| Energy (kJ)   | 358.55| 364.75| 369.88| 375.90|
| Nitrogen content (%) | 1.6| 2.4| 3.2| 4.0|

Conventional H&E staining was performed to observe the histological structure.

**Statistical analysis.** Data were analyzed by SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) and expressed as mean ± SD. Multiple comparisons were performed by analysis of variance (ANOVA) followed by post hoc test (LSD test). P<0.05 was considered to indicate a statistically significant difference.

**Results**

**Effects of protein on healing time.** In each group, 22 rats were modeled successfully (Fig. 1) and the pressure ulcer healing time of each group was measured (Fig. 2). Rats in group A and B needed 21.63±1.12 and 19.27±1.22 day, respectively, for their ulcers to heal. However, the healing time of group C was only 16.36±1.57 days, which was significantly lower than that of group A and B (P<0.05). Furthermore, when compared with group C, a little longer healing time was found in group D (17.58±1.31 days), but without significant difference.

**Effects of protein on pressure ulcer area.** The pressure ulcer area was measured over time. No significant difference was found in the pressure ulcer area among groups after the models were constructed. Seven days after surgery the pressure ulcer area of each group was significantly reduced when compared with that of 1 day after surgery (P<0.05). The same situation applied to 14 days after surgery. Of note was that, at these times, the pressure ulcer area of group C (0.19±0.11 cm²) and group D (0.23±0.07 cm²) was significantly lower than that of group A (0.38±0.10 cm²) and group B (0.33±0.17 cm²) (P<0.05). Group D had a larger pressure ulcer area compared to group C, but without significant difference (Fig. 3).

**Effects of protein on body weight.** The rat body weights of each group were recorded at different times (Fig. 4). Before surgery, there was no significant difference in body weight among the 4 groups, and it was significantly decreased in all
groups after the models were constructed, but again without significant difference among groups. Three days after surgery, all the rat body weights were back to normal, and 7 days after surgery, a significantly higher body weight in groups C and D was found when compared with group A and B at the same time (P<0.05). Fourteen days after surgery, compared to after surgery, the body weight of group A and B was significantly increased, but it was significantly lower than that of groups C and D. Rats in group D had a higher body weight compared to group C, but with no significant difference.

**Effects of protein on ALB and Hb levels.** Changes of ALB and Hb levels over time are shown in Fig. 5, respectively. No significant difference was found in ALB or Hb levels among groups before surgery. From 3 days after surgery, a significant decrease ALB and Hb levels was found in groups A and B when compared with that of before surgery. Moreover, these two levels of group C and D were significantly lower than that of group A and B. No significant difference was found in the ALB or Hb levels between group C and D, however, both of these levels of group C were closer to normal level and the ALB level of group D at 14 days after surgery was obviously a little higher than that before surgery.

**Histological structure changes.** After the pressure ulcer models were formed, the structural integrity of the pressure ulcer tissue was very poor and tissue fracture phenomenon was observed. In addition, broken cell structure occurred (Fig. 6A). Fourteen days after feeding with the different feeds, the damage degree of all rat pressure ulcer tissues was significantly improved. However, the structural integrity of the pressure ulcer tissue of rats in group A and B remained very poor, and the cell structure still displayed a certain degree of damage (Fig. 6B and C). At the same time, the pressure ulcer tissue of rats in group C and D had significantly better structural integrity, and the integrity of the cell structure had significantly improved, and the tissue fracture phenomenon had disappeared (Fig. 6D and E).

**Discussion**

In the present study, the effect of protein level on pressure ulcer healing was studied. The results showed that 14 days...
after the models were constructed, rats with 20% protein content feed had the shortest healing time and the smallest pressure ulcer area. Furthermore, body weight, ALB and Hb levels were much closer to the normal level. H&E staining result also suggested that the pressure ulcer healing degree of rats with 20% protein content feed was much better than the others. These results indicated that adequate protein intake has a positive effect on pressure ulcer healing, while excessive or insufficient protein intake is not conducive to healing. Gautam et al (19) reported that high protein consumption diets may induce disorders and increase the burden on metabolic organs, causing a negative impact on body functions. Excessive protein intake also could lead to overweight, which would increase the burden on the body organs and further delay the healing of pressure ulcers. Our results are consistent with the study by Gautam et al (19). The healing degree of rats with the most protein intake (25%) was lower than that of rats with 20% protein intake, although the difference was not significant.

Protein deficiency has been recognized as an independent risk factor for predicting the development of pressure ulcers (20-22). Hb and ALB levels are two common monitoring indicators (23). Reduction in tissue repair function and decreased immunity may occur when the ALB level is reduced, which eventually leads to an increased risk of pressure ulcer and delay the healing time. In addition, if the Hb level was reduced, decreased oxygen carrying capacity would occur (24), resulting in tissue stress tolerance decrease and excess.

Figure 5. Albumin (ALB) and hemoglobin (Hb) levels of each group at different times after surgery. (A) ALB and (B) Hb levels. *P<0.05 when compared with before surgery within the same group; †P<0.05 when compared with group A and B at the same time.

Figure 6. Hematoxylin and eosin staining of rat pressure ulcer tissues. (A) Pressure ulcer tissue after the model was established. (B-E) Pressure ulcer tissues of groups A-D, respectively, on the 14 day after the models were constructed.
healing of pressure ulcer delay (25). The results in this study has shown that the more protein intake in rats, the faster the Hb and ALB levels increased, indicating that the protein intake helps to increase the Hb and ALB levels and then they further promote the healing of the pressure ulcer. Protein is an essential component in the process of tissue repair, which has the ability to improve the body's resistance and to maintain the water balance between plasma and tissue (26). Long-term protein deficiency is likely to cause hypoproteinemia. As a result, water in plasma may penetrate into tissue and cause tissue edema, and this response eventually leads to weakened tissue repair ability (27). In addition, hypoproteinemia can lead to decreased anti-infective capacity of injured tissue (28). Of course, excessive protein intake can also have a negative impact because hyperglycemia can increase the burden on organs and then delay in tissue repair ability occurs (29,30). In this study, in addition to healing time and pressure ulcer area of the two indicators, H&E staining result also proved the above view point. We also found that ALB level changes were more sensitive than Hb level ones and it could respond more timely to the nutritional status of rats with pressure ulcer. We consider that ALB level changes can be the first step to predict the risk of pressure ulcer, but further investigations should determine the reason for this phenomenon.

In conclusion, the present study suggests that adequate protein intake is conducive to pressure ulcer healing. Protein plays a key role in tissue repair processes, and insufficient or too much protein intake exerts an adverse effect on the pressure ulcer healing. This study has important guiding significance for the clinical treatment of patients with pressure ulcers.

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Availability of data and materials

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

Authors' contributions

ZQ, WZ and YW contributed to the conception and design of the study and drafted the study. YZ analyzed and interpreted the hematoxylin and eosin (H&E) staining. YT, SS and XL interpreted the data of the study and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Hebei General Hospital (Hebei, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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