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The effect of standard, ketogenic, and Western-type diets applied to rats in pressure wound therapy

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Abstract. – OBJECTIVE: This study aimed at investigating the effect of ketogenic and Western diets on pressure wounds.

MATERIALS AND METHODS: This randomized controlled study used 33 male Sprague-Dawley rats. They were randomly divided into the control, ketogenic, and Western diet groups. Pressure wounds were created on the rats' backs.

RESULTS: Wound healing of the Western diet group on day 42 was better than the ketogenic and standard groups. In the microscopic examinations, wound closure, damaged muscle tissue repair, angiogenesis, vascularization, granulation, and collagenization in the Western diet group were faster than in the ketogenic and standard groups.

CONCLUSIONS: The Western diet was potentially effective for pressure wound healing. Future research should be conducted to clarify how this affects the wound-healing process.

Key Words:
Ketogenic diet, Pressure wound, Rat, Western diet, Wound healing.

Introduction

Pressure ulcers are a serious health problem encountered in healthcare services. Studies[1,2] have examined the relationship between pressure wound healing and nutrition. They showed that protein-based diets accelerate the wound-healing process and increase the likelihood of wound closure; however, studies[3] examining the effect of different dietary patterns on pressure wound healing are limited. The ketogenic diet comprises high fat, low carbohydrate, vegetable oil, and sufficient protein. It affects various diseases and has become popular recently. Furthermore, the popularity of the Western diet (with high fat and high carbohydrate content), including refined cereals, snacks, fatty processed meats (red meats), pizza, juices, mayonnaise, nuts, and corn syrup, has increased. Its popularity may be due to the long working hours of both men and women, increasing their likelihood of ordering fast food and consuming cheaper processed foods[4,5].

Some studies[6-8] showed that ketogenic and Western diets have positive and negative effects on health. A study[9] reported that the ketogenic diet increased resistance to brain damage by reducing cortical degeneration in patients with traumatic brain injury. Another study showed that ketone body production reduces cellular damage and injury in neurons and cardiomyocytes[6,7]. Mu et al[10] determined that the ketogenic diet significantly reduced demyelination and attenuated axonal damage in rats following diffuse axonal injury. Moreover, the ketone body β-hydroxybutyrate increases myelination and cell viability and decreases axonal degeneration caused by glucose deprivation and oligodendrocyte death. Viggiano et al[11] determined that the calorie-restricted ketogenic diet reduces capillary density in the cerebral cortex. The Western diet has been identified to exacerbate traumatic brain injury, hinder healing, and reduce responses to treatment[12]. An alternative study showed that the Western diet promoted kidney damage, inflammation, and fibrosis[8]. Several studies showed that ketogenic and Western diets have various effects on health. However, no study in the literature examined the effect of these two diets on pressure wound healing. Therefore, this is the first study to investigate the effect of ketogenic and Western diets on pressure ulcer healing.

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Materials and Methods

Ethical Consideration

All experiments were performed according to the recommendations of the European Community Guide for the Care and Use of Laboratory Animals. The study was approved by the Animal Experiments Ethics Committee of Sakarya University (date: 26/01/2019; number 26) and supported by the Sakarya University Scientific Research Projects Coordinator (No. 2019-7-25-15). We followed the institutional and national standards for the care and use of laboratory animals.

Study Design

This study was conducted in the Laboratory Animal Application and Research Center of Sakarya University, Turkey. We used 33 male Sprague-Dawley rats, aged 6-8 weeks and weighing 100-185 g. A blinded investigator randomly divided the rats into the control (n = 11), ketogenic (n = 11), and Western diet groups (n = 11).

Study Subjects and Groups

Thirty-three male Sprague-Dawley rats were fed determined diets and water ad libitum, housed at 21 ±1°C in a well-ventilated environment, and kept away from noise and vibration devices in a 12-h day/night cycle.

The sample size was determined using Cohen's criteria. Accordingly, within the 95% confidence interval limit (α error probability = 0.05), at 0.80 power (1-β), and in the medium-effect size (Cohen's d estimated effect size, dz = 0.5), the minimum sample size was calculated as 33 (11 rats for each group). Subsequently, the rats were divided further into three groups:

- Standard diet group (control): the rats were fed in single cages for 9 weeks + 3 days with the standard diet (calories consisting of 77.3% carbohydrate, 2.7% fat, and 20% protein; 3,000 kcal/1,000 g) and water *ad libitum*.
- Western diet group: the rats were fed in single cages for 9 weeks + 3 days with the Western diet (calories consisting of 39.70% carbohydrate, 39.51% fat, 19.53% protein, and 1.26% other components; 4,540 kcal/1,000 g) and water *ad libitum*.
- Ketogenic diet group: the rats were fed in single cages for 9 weeks + 3 days with the ketogenic diet (calories consisting of 4.95% carbohydrate, 74.24% fat, 19.53% protein, and 1.28% other components; 5,809 kcal/1,000 g) and water *ad libitum*.

The diets were obtained from Arden Research and Experiment, Ankara, Turkey.

Wound Creation

We used the pressure ulcer rat model developed by Stadler et al[13]. After feeding the rats with their diets, they had been were anesthetized with 100 mg/kg of ketamine (Ketasol, Richterfarma, Austria, i.p.) and 10 mg/kg of xylazine (Rompun, Bayer Ag, Leverkusen, Germany, i.p.) on the first day of the fourth week. The rats' backs were shaved and disinfected with Batticon® (Adeka, Samsun, Turkey). The rats' skin between the two scapulae was gently removed, and two neodymium magnets with a diameter of 15 × 5 mm and a power of 2,000 G (weight: 6.53 g) were placed on both sides of the removed skin. Compression was performed with an 8-h magnet attachment and 8-h release cycles (ischemia-reperfusion model) as seen in the literature (Figure 1), and a pressure wound developed after 72 h. Wound healing was evaluated with macro- and microscopic data. Macroscopic data comprised the wound area measurements obtained manually by a researcher. The wounds were photographed using the same camera with the help of a millimeter ruler on days 0, 7, 14, 21, 28, 35, and 42. Microscopic data were obtained from biopsy specimens excised from the wound site. The rats were sacrificed after the experiment.

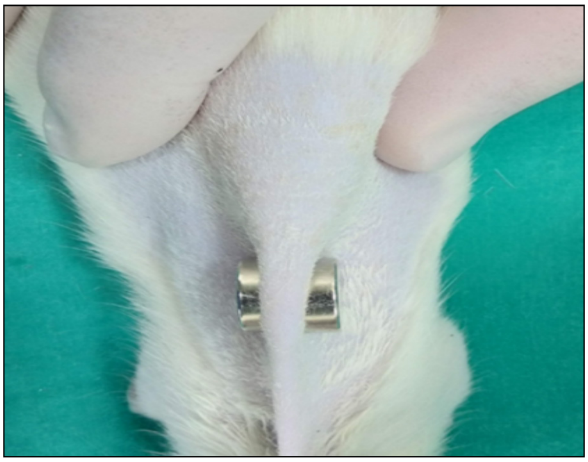

Figure 1. Pressure wound by neodymium magnets.

Hematoxylin-Eosin

Wound tissue was obtained on days 7 and 42. The tissue samples were fixed in 10% buffered formaldehyde for 18 h. To remove the water in the tissue (dehydration), it was passed through 70%, 80%, 90%, 96%, and 100% alcohol series in order and cleared with xylene (Merck 108323). The tissues were prepared for sectioning by keeping them in liquid paraffin in a 56°C oven (Binder) for 2 h and embedding them in a paraffin block. Hematoxylin-eosin (H&E) was used to stain sections of 4-5 microns obtained using a Leica RM 2255 (Leica Biosystems, Nussloch, Germany) microtome. A histologist evaluated the histopathological examinations using a Nikon light microscope, considering the "Wound Healing Score Evaluation Criteria".

Blood Analysis

After the experiment, blood was obtained from the rats' tails to measure interleukin 6 (IL-6) and Ki-67 levels. Measurements were performed after transferring blood into gel tubes. Subsequently, blood was centrifuged at 3,000 rpm for 10 min and turned into the serum. The serums were transferred to Eppendorf tubes and kept at -80°C until analysis. The serums were analyzed using an autoanalyzer device working with commercial kits [Rat IL-6 ELISA kit (YLA0031RA); Rat Ki-67 protein ELISA kit (YLA0801RA)].

Data Collection

Data were grouped according to their blood and macroscopic and microscopic results. Blood data included IL-6 and Ki-67. The photos were transferred to a computer, and the area was calculated using the AUTOCAD program. In the microscopic evaluation, a pathologist blinded to the study examined re-epithelialization, angiogenesis, collagen, granulation, fibroblasts, proliferation, hair follicles, and adipose tissue. Microscopic evaluation of the pressure wound was performed on days 7 and 42.

Statistical Analysis

Statistics were presented as number, median, and standard deviation. Parametric tests were used to evaluate the measurements with normal distributions. Thus, one-way analysis of variance was used to determine the differences between more than two groups compared with quantitative data used to obtain values with normal distributions. The Kruskal-Wallis' H test was used for more than two groups of data not fitting the standard distribution patterns. The Bonferroni multiple comparison test was used to determine the different groups in the data tested using the analysis of variance. Similarly, the Bonferroni corrected multiple comparison test, which is different from the Kruskal-Wallis H test, was performed. The Friedman test was used for repeated measurements due to the low quantity of available data, without searching for a normal distribution to examine the time factors of their averages. The groups presenting differences were analyzed using the multiple comparison test. Spearman's correlation was also used. A p-value of <0.05 was accepted as statistically significant.

Results

IL-6 and Ki-67

In the ninth week, the serum IL-6 levels were 1.62±1.16, 1.86±0.75, and 1.90±0.59 in the ketogenic, standard, and Western diet groups, respectively. The serum Ki-67 levels were 12.84±0.18, 12.06±1.52, and 11.97±1.73 in the ketogenic, standard, and Western diet groups, respectively. No difference was found in the levels of IL-6 ($F = 0.338$, $p = 0.716$) and Ki-67 ($F = 0.892$, $p = 0.420$) between the groups.

Wound-Site Measurement

The wound-site measurements of the ketogenic, Western, and standard diet groups were not statistically significantly different from the measured groups on days 0, 7, 14, 21, 28, and 35 ($p > 0.05$). However, they were statistically significantly different on day 42 ($p < 0.05$). In addition, the wound measurements of the rats fed with the Western diet were significantly smaller than those fed with the ketogenic diet. Accordingly, wound healing of the Western diet group on day 42 was better than the ketogenic and standard groups (Figure 2).

Microscopic Examinations

Microscopic examinations showed that wound closure, damaged muscle tissue repair, angiogenesis, vascularization, granulation, and collagenization in the Western diet group were faster than in the ketogenic and standard groups. The Western diet group had faster formation and recovery results than the ketogenic and standard diet groups (Figure 3A, Figure 3B, Figure 3C, Figure 4A, Figure 4B, and Figure 4C). In general, wound healing in the standard group was slower than in the ketogenic and Western diet groups.

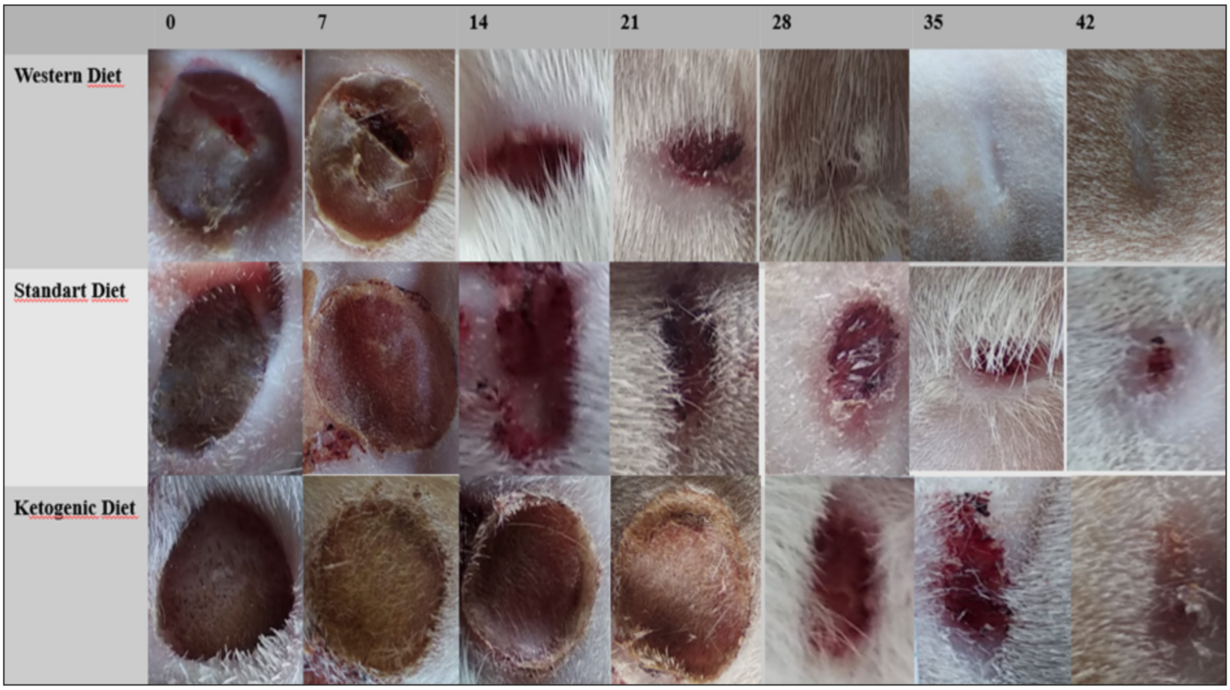

Figure 2. Wound photos (at 0, 7, 14, 21, 28, 35 and 42 days) of the rats fed with Western diet, standard diet and ketogenic diet

In the ninth week, no significant relationship was found among IL-6 and Ki-67 levels, tissue re-epithelization (r = -0.03, p = 0.85; r = 0.12, p = 0.50), granulation tissue (r = 0.08, p = 0.65; r = 0.11, p = 0.51), collagen accumulation (r = -0.08, p = 0.67; r = 0.07, p = 0.71), inflammatory cells (r = 0.00, p = 0.98; r = 0,06, p = 0.72), angiogenesis (r = -0.04, p = 0.81; r = 0.12, p = 0.50), and ulcers (r = -0.22, p = 0.21; r = -0.02, p = 0.92).

Discussion

In this experimental model, the wound healing process between the groups was compared using

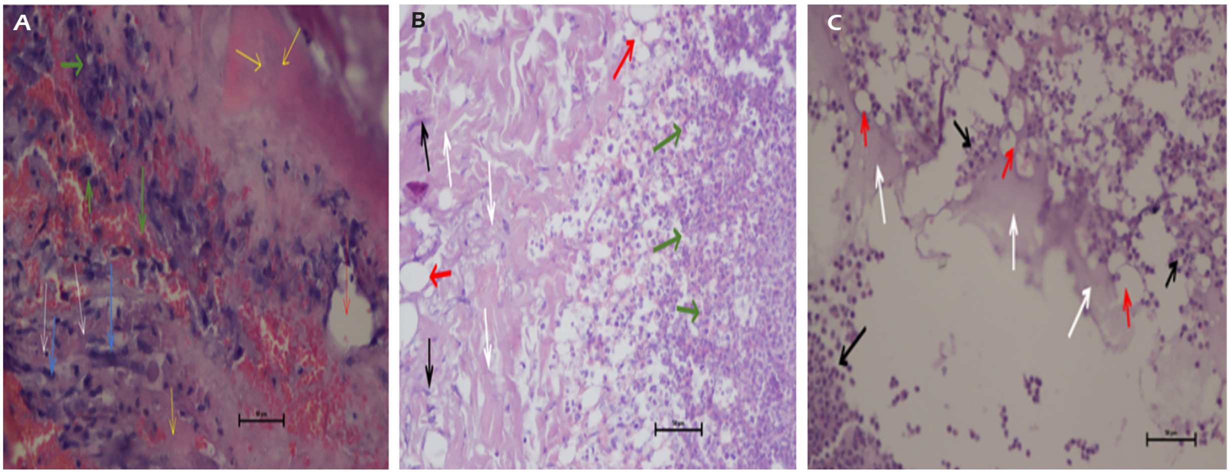

Figure 3. A, Day 7 of the pressure ulcer tissue in ketogenic diet group: collagenization (*white arrow*), fibroblast (*blue arrow*), blood vessel (*red arrow*), granulation, bleeding area (*green arrow, red area*), muscle (*yellow arrow*) (Haematoxylin Eosin staining, *20). **B**, Day 7 of the pressure ulcer tissue in western diet group: collagenization (*white arrow*), fibroblast (*black arrow*), blood vessel (*red arroaw*), granulation (*green arrow*) (Haematoxylin Eosin staining, *20). **C**, Day 7 of the pressure ulcer tissue in standard diet group: blood vessel (*red arrow*), bleeding area (*white arrow*), granulation (*black arrow*) (Haematoxylin Eosin staining, *20)

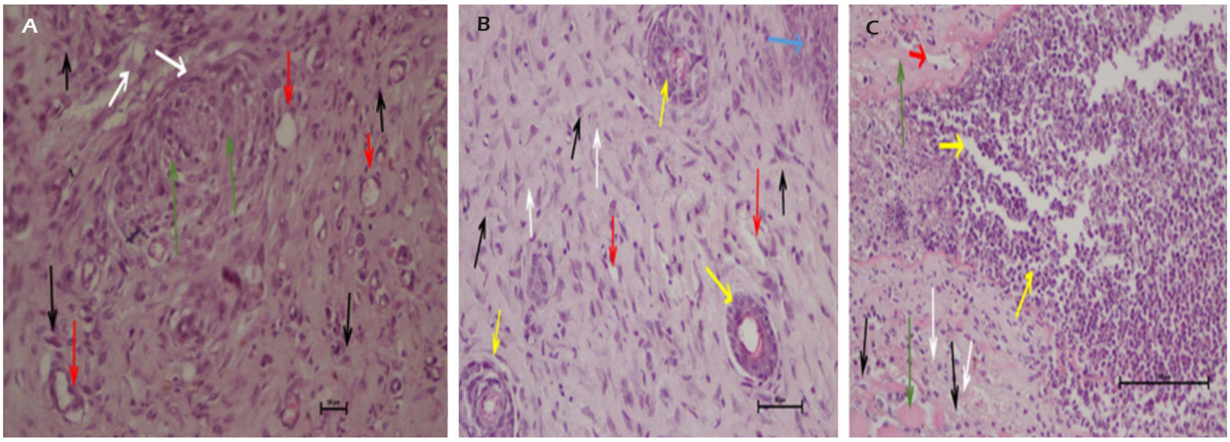

Figure 4. **A**, Day 42 of the pressure ulcer tissue in ketogenic diet group: collagenization (*white arrow*), fibroblast (*black arrow)*, blood vessel (*red arrow)*, granulation (*green arrow*) (Haematoxylin Eosin staining, *20). **B**, Day 42 of the pressure ulcer tissue in western diet group: epithelization (*blue arrow*), collagenization (*white arrow*), fibroblast (*black arrow*), blood vessel (*red arrow*), hair root (*yellow arrow*) (Haematoxylin Eosin staining, *20). **C**, Day 42 of the pressure ulcer tissue in standard diet group: collagenization (*white arrow*), fibroblast (*black arrow*), blood vessel (*red arrow*), granulation (*yellow arrow*), muscle (*green arrow*) (Haematoxylin Eosin staining, *20).

macroscopic and microscopic wound evaluations. On day 42, the wound sites of the Western diet group were significantly smaller than the ketogenic diet group.

The histopathological examinations also determined that the pressure wound healing in the rats fed with the Western diet significantly improved more than those fed with the ketogenic and standard diets. Furthermore, the wound healing of the rats fed with the ketogenic diet was better than those fed with a standard diet. In the literature, no studies have examined the effect of the Western diet on pressure wound healing. From the literature review, the Western diet maintains nitrogen balance levels, which affects the wound healing process and collagen levels, resulting in faster entry into the granulation stage and increasing energy levels by increasing transforming growth factor β (TGF-β) and platelet-derived growth factor, which are known to increase the collagen content in the wound site in the early stages of healing. Although collagen content increases oxidative stress levels, vascular cells can adapt to this situation because it contains the energy needed in the wound healing process. Endogenous insulin production is caused by glucose, which increases due to high carbohydrate levels, reduces protein catabolism with anabolic effects, and increases new protein production. Carbohydrates are found in keratinocyte cells in the epidermis layer and in the structure of connective tissue proteins, such as collagen and elastane, to which oils provide resistance against external factors. Cells and their membranes are the basic building blocks, and prostaglandin, also known as local hormone (which plays a role in inflammation, hemostasis, and platelet function), acts as a precursor agent of hormone-like eicosanoid compounds, such as thromboxane and leukotrienes, which are involved in coagulation. Compared with the rats fed with other diets, this result may positively affect wound healing[14-17].

Furthermore, casein, found in Western and ketogenic diets, increases fibroblasts, keratinocytes, and serotonin which makes cell migration and cell survival increasing. Melatonin, a strong antioxidant that stimulates epithelial migration, increases the proliferation phase, fibroblast density, angiogenesis, VEGFR2 associated with angiogenesis, and antioxidant enzyme levels. It also induces IL-1, tumor necrosis factor α, and TGF-β production and reduces inflammation and necrosis. Thus, compared with the standard diet, the Western and ketogenic diets have positive effects on the wound healing process[18-20].

Furthermore, the ketones from the ketogenic diet prevent mitochondrial dysfunction-mediated oxidative stress by providing alternative substrates and exhibiting antioxidant properties. The ketone body containing β-hydroxybutyrate is a natural energy substrate that increases the rate of wound healing, and oral ketone supplements increase the likelihood of wound closure due to blood flow and the provision of alternative energy substrates without dietary restrictions[21,22]. Viggiano et al[11] reported that a calorie-restricted ketogenic diet reduced the capillary density in the cerebral cortex. However, this diet was not used in this study.

Limitations

The lack of molecular data limits the present study. Studies that use molecular data to examine the effect of Western and ketogenic diets on wound healing are required.

We could not report the rats' average daily calorie consumption in the three groups. The animals were fed *ad libitum*. Any difference in calories intake could affect wound healing. Therefore, other studies are required to clarify this observation.

Conclusions

Based on macroscopic and microscopic evaluations, the Western diet was potentially effective in wound healing, which appears to accelerate the wound healing process. Considering the photos of the wound, the wound-healing process of the Western diet group was better than the ketogenic and standard diet groups on day 42. In the microscopic examinations, wound closure, damaged muscle tissue repair, angiogenesis, vascularization, granulation, and collagenization in the Western diet group were faster than in the ketogenic and standard groups. Moreover, IL-6 and Ki-67 did not affect the wound-healing process.

Further studies comprising larger groups and human subjects and examining the mechanism of the Western diet on the wound healing process are highly recommended. In addition, future studies using molecular data should examine the effect of the Western diet on the wound-healing process.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

This study is derived from a doctoral thesis.

Ethics Approval

The Animal Experiments Ethics Committee of Sakarya University approved this study (date: 26/01/2019; number 26).

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Data Availability

The data used for publication can be accessed through the corresponding author.

Authors' Contribution

Conceptualization, Ç.Ö.S, S.H. and Ç.H.; methodology, Ç.Ö.S, S.H. and Ç.H.; formal analysis, Ç.Ö.S and SH.; data curation, Ç.Ö.S, S.H. and Ç.H.; writing-original draft preparation, Ç.Ö.S, S.H. and Ç.H.; histology, B.Ö.; writing-review and editing, Ç.Ö.S, S.H., B.Ö., Ç.H.; supervision, S.H. and Ç.H.; project administration, Ç.H. and S.H.; funding acquisition, Ç.Ö.S, S.H., and Ç.H. All authors have read and agreed to the published version of the manuscript.

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