

Histological investigation of the effects of western diet on pressure wound healing period

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ABSTRACT

Objective: The stages of skin wound healing are a dynamic process and it is thought to be related to nutrition. Carbohydrates, proteins and fats have particular importance in different periods of recovery process. Our study has aimed to examine the effects of a western diet with high protein, fat, and carbohydrate content on pressure ulcer healing.

Material and Methods: In this study, we used 22 healthy male Sprague Dawley rats weighing 100-185 g. We randomly divided the rats into two groups. The rats were fed according to the indicated diets (standard diet and western diet). On the first day of the fourth week, ischemia skin by histopathological examinations of the wound tissue samples on the 7th and 14th days of the wound healing period.

Results: Statistically significant differences were observed in histological and immunohistochemical parameters in the tissue samples on the 7th and 14th days. On the 7th day, there were re-epithelialization (P=0.003), granulation cell density (P=0.004), inflammation (P=0.004), and angiogenesis (P= 0.003). We found re-epithelialization (P=0.001), granulation cell density (P=0.002), inflammation (P=0.002), and angiogenesis (P=0.001) on the 14th day. On the 7th and 14th days, we found the p-value between Ki-67 immunohistochemical staining percentages as P= 0.003 and the p-value for VEGF as P=0.002.

Conclusion: We determined that in short-term wound healing, the western type diet was more effective on pressure wound healing than the standard diet.

Keywords: decubitus ulcer, western diet, wound healing

INTRODUCTION

A decubitus ulcer is defined as the skin or tissue damage generally on the pointed parts of the bones caused by both pressure and compression or only by force (1). In addition to the more frequent observations of decubitus ulcers generally on the pointed parts of the bones, they could also be caused by the medical equipment, which leads to compression, pressure or friction such as orthopaedic casts, cartridges, catheters, and compression machines (2). Nearly 4 % of decubitus ulcer cases need hospital care to treat thoroughly. This treatment also requires a substantial amount of money (3).

The dermal wound healing is an active process. It includes the phases of inflammation, proliferation, and the reformation of the skin into its old state. In the inflammation phase, cell migrations, cytokines, and growth factors play an essential role. This is sovereign in the stimulation of the vascular proliferation; thus, the tissues' reformation occurs (1). The studies conveyed indicate that wound healing connects with nutrition by nature. In wound healing and other cases requiring treatment, insufficient nourishment affects the recovery period negatively. This situation, which is derived from especially the lack of protein and energy, is also corroborated by the clinical results (4).

The most energy-requiring phase in wound healing is the collagen synthesis phase. In the wound healing period, if there isn't enough carbohydrate intake, other energy sources such as proteins can be used as the primary source, leading to the limitation of wound healing (1).

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Insufficient carbohydrate intake may substantially set wound healing back by causing the inflammation phase to drag out. It is thought that increasing the protein intake in wound healing or sickness period may ensure a shorter reparation of the tissue damage (2). The role of fats in wound healing has not been studied in detail (3, 5). Nevertheless, it is known that there has been a rising need for essential oils during traumatization.

The Western diet is a diet with low consumption of vegetables and beverages with high protein content (mainly processed meats), saturated fats, refined grains, sugar, alcohol, salt, and corn syrup (6). Living conditions in daily life have allowed western type nutrition to be preferred widely, especially among the working population. In such a context, we aim to study the effects of the western diet with high protein, fat, and carbohydrate content on pressure ulcers the decubitus ulcer wound healing.

MATERIALS and METHODS

This study has been conducted by Sakarya University Animal Testing Local Ethics Committee approval in Sakarya University Experimental Research and Medical Centre (Dated 05/08/2020 and numbered 46). The procedures on the rats are humane, and the standards of the study are pertinent to the standards of the existing ethical animal testing procedures.

Experimental Animals

In this study, 22 healthy male Sprague Dawley rats weighing between 100-185 grams were used as experimental animals. This experiment has been conducted in Sakarya University Experimental Research and Medical Center (Sakarya, TURKEY). The rats were fed with water and ad libitum according to the determined diets (standard diet and western diet). Rats were kept at 21 ± 1 °C in well-ventilated places within 12- hour day-night cycle. Animal diets, both the standard and the western type, were produced by the experts at a private corporation (Arden Research& Experiment, Ankara, TURKEY).

Experimental Protocol

The 22 rats were randomly divided into two groups, 11 each in the control and western diet groups, which would be statistically significant. Rats were fed with a Standard diet (calories 77.3% carbohydrate, 2.7% fat, and 20% protein) and a western diet (calories 39.70% carbohydrate, 39.51% fat, 19.53% protein, and other components 1.26%) according to the groups.

The decubitus ulcer model has been used by Stadler et al. (7). After feeding procedure, the rats were anesthetized by the Ketamine HCL 100 mg/kg IM (Ketasol% 10 10 ml) (Ketasol, Richterfarma, Austria) and then by Xylazine 10 mg on the first day of the fourth week. The hair between the two blade bones of the rats was shaved. The skins between the two blade bones of the rats in all groups were gently removed. Two neodymium magnets with 15x5 mm in diameter and 2000 Gauss in power (6.53g in weight) were implemented into both sides of the removed skin.

Compression was applied in 8-hour magnet fastening and 8-hour release (ischemia-reperfusion model), just as in the literature and the decubitus ulcers were developed after 72 hours (7). The rats were fed identical diets until the end of the study.

Histopathological Evaluation

Tissue samples were collected from wounds on the seventh and fourteenth days of the decubitus ulcers. The tissue samples were immobilized in the formaldehyde buffered 10% for 48 hours. The tissue sample was soaked in paraffin-embedded blocks after the tissue embedding processes. The 4–5-micron incisions taken by the Leica RM 2255 microtome were processed in hematoxylin-eosin staining. According to “The Wound Healing Points Evaluation Criteria”, a histologist evaluated the histopathological examinations via a Nikon model light microscope according to “The Wound Healing Points Evaluation Criteria”. A score of 0–3 was given to each section according to the presence of inflammatory cells and the levels of angiogenesis and epithelialization as previously described by Sedighi et al., 2016 (8) with minimal modifications (Table1).

Immunohistochemical Staining Method

The tissue samples, which were cut in 4 microns from the paraffin-embedded blocks, were deparaffinised and got through decreasing alcohol series. The preparations in citrate buffers went under heat treatment for 20 minutes in the microwave. After that, all incisions were blocked in 3% of H₂O₂ by endogen peroxidase- activity. Primary antibodies were VEGF (Genetex), and Ki-67 (Genetex), which were used in a 1/300 ratio, and then secondary antibodies (Ultra Vision Large Volume Detection System Anti-rabbit by Lab Vision, HRP) were used. Each step was implemented by the producing company’s procedures. Diaminobenzidine (DAB) was used to make the paint visible. Mayer’s hematoxylin was used for contrast colouring. The preparation was covered by the mounting medium (Aqueous Mounting Medium by Scy Tek). As a result of Ki-67 and VEGF staining, the preparations were scored in randomly chosen five staining areas and the area with the highest score was determined. In both groups, at least 100 cells were marked within each x40 magnifying area. In incisions, the percentage of the stained cells and staining level were the criteria to be chosen. For each incision, Immunohistochemical staining scoring was calculated according to H-SCORE, which is a scoring algorithm formulated as $(I \times PC)$, (I: the level of staining, PC: the percentage of stained cells in each level) (9).

Statistical Analysis

Statistical analyses were performed using the SPSS 24.0 package program (SPSS Inc. and Lead Tech. Inc. Chicago, USA). Shapiro Wilks test was used in compliance with normal distribution. Kruskal Wallis test was used for numerical data of the subgroups that did not show normal distribution. Intergroup evaluations for statistically different parameters were performed using the Mann-Whitney U test and comparing them in pairs. Results were given as mean \pm standard deviation. For all statistical analyses, a two-tailed P-value <0.05 was considered statistically significant.

Table 1. Histological scoring parameter of epithelialization, angiogenesis, granulation tissue formation, and inflammatory cells.

Parameter/Score	0	1	2	3
Inflammatory cells	1–5 inflammatory cells per histological field	5–8 inflammatory cells per histological field	8–11 inflammatory cells per histological field	11–15 inflammatory cells per histological field
Reepithelialization	Absence of epithelial proliferation in $\geq 70\%$ of tissue	Incomplete epidermal organization in $\geq 50\%$ of tissue	Moderate epithelial proliferation in $\geq 60\%$ of tissue	Complete epidermal remodeling in $\geq 80\%$ of tissue
Angiogenesis	Absence of angiogenesis including congestion and hemorrhage	2–4 vessel per site, congestion and hemorrhage	4–6 vessel per site, slight congestion	7–8 vessel per site vertically disposed towards the epithelial surface
Granulation cell density	None, completely Disorganized and distorted	Minimal/immature thin	Mild/moderately mature granule layer	Evident/ Thick, $\geq 80\%$ organized

RESULTS

Re- epithelialization

In standard and Western diet groups, when the tissue samples from the 7th and 14th days were compared in terms of re-epithelialization, statistically significant differences between the tissues of those two days were observed (P-value in order is $p=0,003$; $p=0,001$). When the tissue samples of standard diet groups from the seventh and fourteenth days were compared in terms of re-epithelialization, statistically significant differences between the tissues of those two days were observed ($p=0,002$). In the fourteenth day samples, better re-epithelialization was observed. In terms of re-epithelialization, statistically significant differences between the tissue's samples of the seventh and fourteenth days were observed in the Western diet group ($p=0,001$). In the fourteenth day samples, better re-epithelialization was observed (**Image 1**).

Granulation Cell Density

When the standard and Western diet groups were compared on the 7th and 14th days in terms of granulation cell density, there were statistically significant differences in terms of granulation cell density in favour of the western diet group on both the 7th and the 14th days (P-value in order was $p=0,004$; $p=0,002$). The granulation cell density in the standard diet tissue samples was observed to be better on the 14th day than on the 7th day ($p=0,001$). Under the same conditions of the western group, statistically significant differences were recorded on the 14th day compared to the 7th day ($p=0,000$) (**Image 1**).

Inflammation

When the 7th day and the 14th-day results of standard and western diet results were compared in terms of inflammation cell density, on both dates, the inflammation cell density had statistically significant differences in favour of the diet of the west group. The P values on the 7th and the 14th days were respectively $p=0,004$ and $p=0,002$. The tissue samples of the standard diet were observed to have statistically significant differences on the 14th day than on the 7th day ($p=0,001$). The inflammation cell density of the Western diet group's tissue samples on the 14th day had a statistically significant difference compared to the 7th-day samples ($p=0,000$). Under the same conditions, it was in favour of the western group on the 14th day ($p=0,000$) (**Image 1**).

Angiogenesis

When the standard and Western diet groups were compared on the 7th and 14th days in terms of angiogenesis ratio, there were statistically significant differences in favour of the western diet group on both the 7th and the 14th days (P-value in order was $p=0,003$; $p=0,001$). The angiogenesis ratio in the tissue samples of the standard diet was observed to have statistically significant differences on the 14th day than on the 7th day ($p=0,002$). The angiogenesis tissue samples of the western diet were honoured to have statistically significant differences on both the 7th day and the 14th day ($p=0,000$) (**Image 1**).

Immunostaining Results

The tissue samples of standard and Western diet groups on the 7th and the 14th days were separately stained, and the results were evaluated.

Ki-67 Immunostaining

When the standard and Western diet groups were compared on the 7th, there was a statistically significant difference between the two groups ($P=0,000$). The percentage of the Ki-67 immunostaining was observed to be relatively high in the western group. When the standard and Western diet groups' results were compared on the 14th, there was a statistically significant difference in the percentage of the Ki-67 immunostaining between the two groups ($P=0,000$). The rate of the Ki-67 immunostaining in the Western group on the 14th day was observed to be relatively low (**Image 2**).

VEGF Immunostaining

When the standard and Western diet groups were compared on the 7th regarding the percentage of the VEGF immunostaining, there was a statistically significant difference between the two groups ($P=0,000$). The rate of the VEGF immunostaining in the western group was observed to be relatively high. When the standard and Western diet groups' results were compared on the 14th, there were statistically significant differences in the percentage of the VEGF immunostaining between the two groups ($P=0,000$). The rate of immunostaining in the Western group on the 14th day was observed to be lower (**Image 3**).

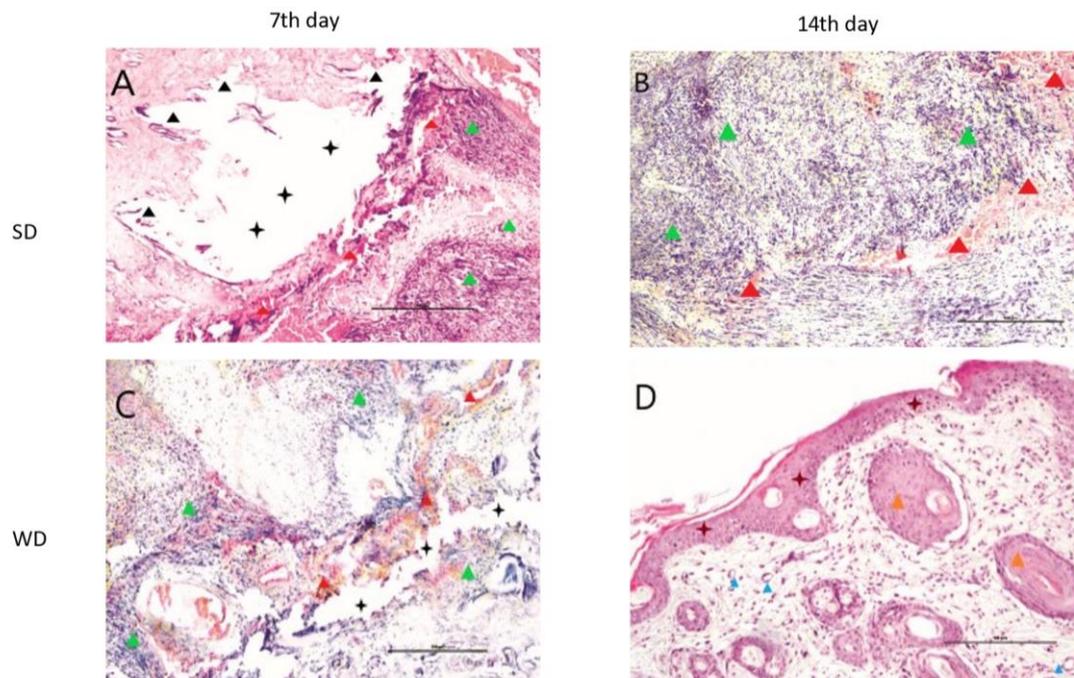


Image 1. 7th and 14th-day scar tissue samples from SD and WD nutrition groups. X100, 100 scale bar (Hematoxylin Eosin). Large wound areas were seen in the 7-day examples in the SD (A) group, and dense granulation areas were seen in the 14th-day samples (B). Small wound areas and bleeding areas were seen in the 7th-day models (C) of the WD group. It was observed that epithelialization was completed, and healing was completed in the 14th-day samples (D). SD: Standard diet group, WD: Western diet group, black star: wound area, red arrowhead: bleeding area, green arrowhead: granulation area, black arrowhead: damaged muscle area, blue arrowhead: blood vessel, orange arrowhead: hair root, burgundy arrowhead: epithelial layer.

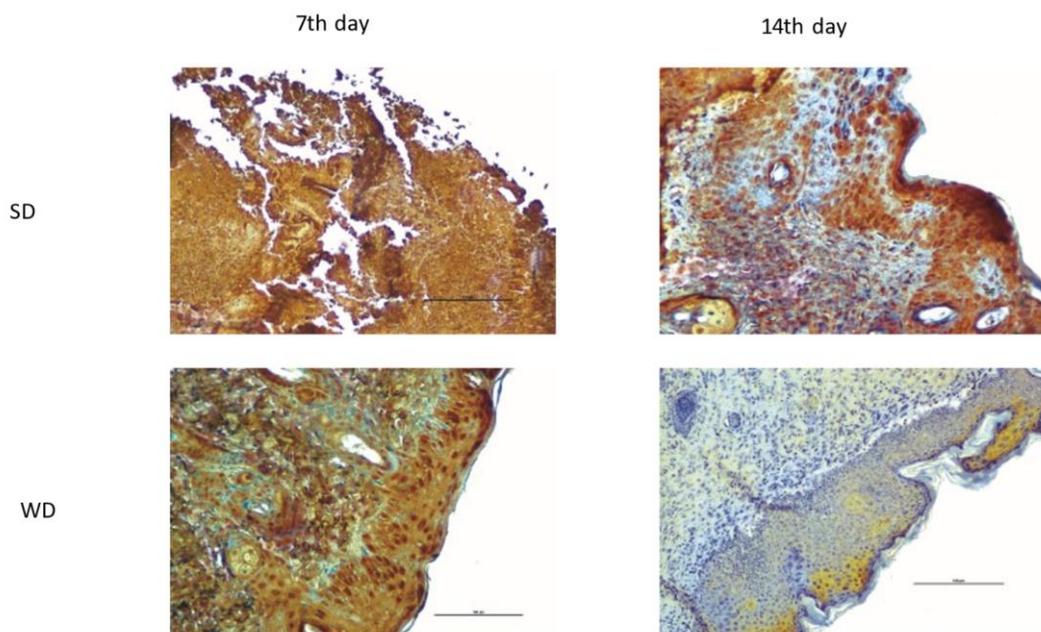


Image 2. Ki-67 immunoreactivity preparations in the wound tissue samples of the SD and WD groups on the 7th and 14th days. X100, 100 scale bar. It was seen that Ki-67 activity in the 14th-day models in the WD group was significantly lower than in the SD group. In the tissue samples on the 7th day, more intense Ki-67 immunoreactivity was observed in the SD group than in the WD group. The more intense Ki-67 immune reactivity in the SD group indicates that the healing process is slower in the acute period. SD: Standard diet group, WD: Western diet group.

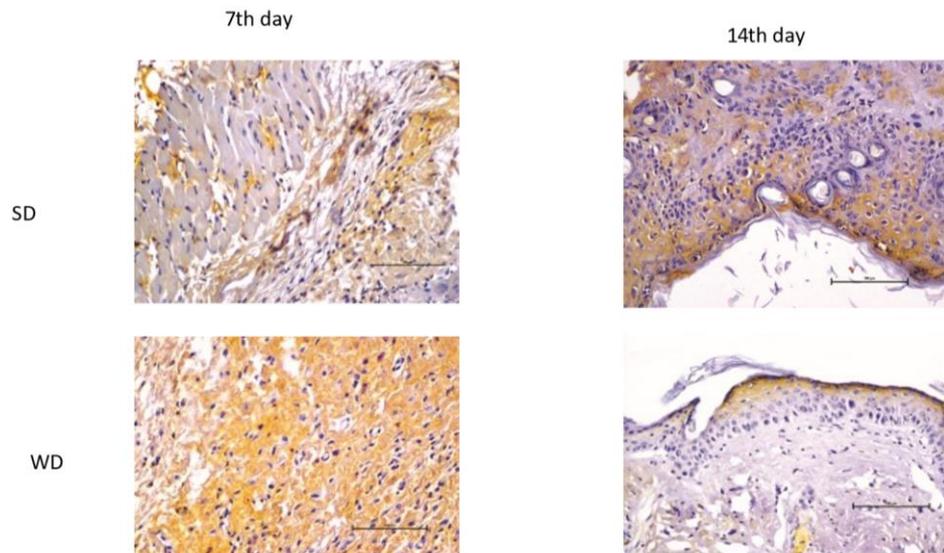


Image 3. VEGF immunoreactivity preparations in the wound tissue samples of the SD and WD groups on the 7th and 14th days. X100, 100 scale bar. It was observed that VEGF immune reactivity in the WD group was less intense in the samples on the 14th day compared to the SD group, and the wound healing process was completed at the end of the 14th day. SD: Standard diet group, WD: Western diet group.

DISCUSSION

Decubitus ulcers are related to ischemia-reperfusion injury (10, 11). The physical movements on the skin tissues lead to blood vessel pressure and, therefore they cause ischemia. When the pressure effect decrease or increase, and the decompression on the vessels, the re-oxygenation of the tissues may induce ischemia-reperfusion injury (12). For this reason, to understand the mechanisms in the formation and the rehabilitation of decubitus ulcers, the dynamics of blood vessel formation need to be researched. In our study, we have used the ischemia-reperfusion model of Stadler et al. (7). Vascular endothelial growth factor (VEGF) underlies vascularization (13-15). VEGF can be related to cell regeneration, granulation tissue formation, and reformation (16, 17). Ki-67 is an important nucleoprotein, which marks the proliferative cells defined as proliferation indicator (18-21). Our study used Ki-67 to show the cell formation density of the newly forming cells with its VEGF effect on the wound area since the beginning of neovascularization. In our study, the tissue samples were collected from the Western Diets, and Standard Diets applied rats' wound area on the 7th and 14th days. We have identified the density of VEGF and Ki-67 in our tissue samples as semiquantitative (H-score). In addition to this, we have used the histological markers, which indicate the stages of wound healing such as inflammatory cell density, granulation tissue, angiogenesis and re-epithelialization while examining the preparation stained with H.E. on the 7th and 14th days.

The formation of granulation tissue is the most significant indicator of wound healing (22). In the appearance of the wound, the fibroblasts, the collagen sarcostyles and the capillary vessels between the wound lips colligate the wound lips by becoming parallel to the wound surface (23). In the last phase of wound healing, the re-formation is characterized by the new Epithelialization and scar tissue formation (24).

In our study, dense blood vessels (angiogenesis) and granular cell accumulation in the tissue samples which were collected from the Western Diets group and stained by H.E. on the 7th day were observed more in comparison with the 7th-day tissue samples from Standard Diets group.

This observation leads to the idea of W.D.'s acceleration of the wound healing process. It was observed that the angiogenesis and the inflammatory cell accumulation in the preparation of the tissue samples taken from the rats fed on W.D. lowered significantly in comparison to the S.D. group on the 14th day. It was observed that there were more intense spots of fibroblast in the WD-14th-day samples than 7th-day samples. Concordantly, it was observed that the fast-healing process continued, and the formation of folliculitis and epithelialization started to be seen in the Western Diets group. When microscopically examined, the vascularization, granulation, and collagenases spots were much slower in the standard group than in the western group.

In this study, the percentage of Ki-67 and VEGF immune expression was higher in WD than SD in the 7th-day samples. Also, it was observed that the percentage of Ki-67 and VEGF immune expression was higher in SD than WD in the 14th-day samples. It is remarkable that the intensities of Ki-67 and VEGF immune expression show an increase and decrease in line with the preparation, which indicates the wound healing stages in the HE stained samples. Thereby, in our study, we have observed that WD can affect the completion of cellular regeneration in shorter periods than SD due to its cell proliferation in the wound healing areas.

There are studies in literature stating that a high fat diet impedes wound healing on the dermis (25-27). In those studies, the importance of the diet combination used in the healing process on wound healing is emphasized.

Until nowadays, the studies examining especially the effect of WD on decubitus wounds' healing process frequently have been found. The studies on high-fat and sugar diets are conducted more frequently. In one study, it is reported that high-fat diets are effective on collagen production and wound repair and this effect is due to its contribution to the wound healing process rather by changing the nitrogen balance (28). In the mentioned study, they got similar results to our study. There are also other studies with different results from our study in literature. Paulino (2011) reported that the animals fed on a high-fat diet have a delayed wound closure due to long term inflammation after seven days from the formation of the wound (26).

Vascularization is an important factor in wound healing owing to the fact that it provides oxygen and nutrition for cell metabolism (29-31). In a different study from ours, they reported that in mice, a high-fat diet caused a decrease in VEGF expression and, therefore, decreased tissue vascularization in wound healing (27, 32).

CONCLUSION

Frequently consumed WD diet causes renal failure, obesity, high blood pressure, the aggravation of colitis symptoms, the shortening of the colon, the increase in tumorigenesis, the increase in insulin resistance, the development of hepatosteatosis (33). In the wound healing stage, it is suggested that approximately 20-25 % of the total calorie should be given as protein (34). In general, it is advised that the suggested amount of protein in wound healing stages or other wound stages should be given more than the amount needed (35). For that reason, we think that if WD becomes a continuous preference, it can lead to a severe health problem; however, it is suitable for short-term use in the early periods of the wound healing stage due to the need for high protein intake.

Author Contributions: ÖB, HÇ: Project design, Review of the literature, Data collection ÖB: Writing and Revisions

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Ethical approval: All procedures performed at each stage of the study were carried out in accordance with the rules specified in the ethics committee directive.

Conflict of interest: The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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