Original Article

Short-term Effects of Surgical Sterilization on Urinary PH and Specific Gravity, Blood Urea Nitrogen, Serum Creatinine, and Urinary Protein to Creatinine Ratio in Sexually Intact Male and Female Dogs

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ABSTRACT

Surgical sterilization is a widely accepted method to reduce the birth of unwanted dogs and reducing the incidence of reproductive diseases. However, the sterilization of domestic dogs has generated debate among veterinarians and owners about its effects on urinary parameters. The purpose of this study was to evaluate the short-term effects of surgical sterilization on urinary parameters in healthy adult sexually intact male and female dogs. Ten healthy adult sexually intact mongrel dogs (5 males and 5 females) weighing 15 to 20 kg participated in this study. Only those dogs which were at least 1yearold included in this study to ensure that they had reached sexual maturity. If a dog's urine sample is abnormally colored or milky, has a pH greater than 7.5, and a positive bacterial culture is confirmed, the dog will be excluded. Physical, CBC, and biochemical examinations, such as measurement of blood urea nitrogen (BUN) and serum creatinine (Cr) concentrations and urinalysis (including determination of urine pH and specific gravity (USG) measurement, calculation of the urine protein/ creatinine ratio (UPC), and dipstick evaluation) were performed on each dog. At least 15 days after the sterilization surgery, the dogs underwent physical examination, CBC, biochemistry, and urinalysis at least 15 days later. Urine samples were collected by cystocentesis and stored at 4°C for analysis. After centrifugation at 3000 g for 5 minutes, the urinalysis was performed within 4 hours of collection. There were no significant differences (P>0.05) between the mean BUN and Cr, UPC, USG, and urine pH before and after ovariohysterectomy and castration. The study found that castration and ovariohysterectomy did not affect short-term urinary parameters in healthy adult sexually intact male and female dogs. However, it is uncertain how sterilization affects urinalysis results in sexually intact dogs, and more research with larger sample sizes is needed to determine the impact.

Keywords: Dogs, Female, Male, Surgical sterilization, Urinalysis

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1. Introduction

Surgical sterilization is a widely accepted method for reducing the birth of unwanted dogs and reducing the incidence of reproductive diseases. However, the sterilization of domestic dogs has generated debate among veterinarians and owners regarding its effect on urinary parameters. Gonadectomy is the appropriate term to use when referring to either ovariohysterectomy (OHE; spay) or castration (neutering), as both methods involve the removal of the gonads (ovaries or testes) for surgical sterilization (1). A complete urinalysis quickly provides a wide range of semiquantitative biochemical information such as glucose, protein, albumin, pH, bilirubin, and ketones using urine test strips. Many factors affect urine test results, including age, gender, diet and environment. In addition, a complete urinalysis helps diagnose non-urinary disorders such as bacterial cystitis, protein-losing nephropathy, and transitional cell carcinoma. Endocrine disorders such as diabetes and other systemic diseases such as intravascular hemolysis can also be detected by urinalysis (2). Genital surgery covers a wide range of clinic visits and numerous procedures in pet animals. The hormonal sources that control reproduction in males and females and determine secondary sexual characteristics are eliminated by spaying or neutering. Although one of the primary goals of spaying or neutering is to prevent reproduction, it may also have other physical and behavioral effects. It is not possible for anyone to accurately predict the outcome of spaying or neutering. However, the available research has led to some generalizations about the clinical significance of specific risks and benefits of neutering. Sterilization is generally recommended for all non-breeding animals, because of the prevention of certain diseases. The benefits and risks of castration for male dogs are about the same. There is also conflicting evidence about the risks and benefits of sterilizing animals before six months old. Therefore, it is not possible to strongly recommend or reject this surgery. However, it is clear that spaying bitches before the first heat is better than spaying them after the first heat (3). The appropriate age for ovariohysterectomy is six months, but this surgery is also performed at a younger age, called early sterilization or prepubertal spaying. Breast tumors are rare in male dogs but are among the most common in females. Approximately 35-50% of dogs' mammary tumors are malignant. The primary cause of breast tumors is unknown, but many of them are hormone- related, and most of them can be prevented by spaying or neutering animals before one year of age. The risk of mammary tumors in bitches is 0.05% if OHE is performed before the animal's first heat. The risk of occurrence after the first and second estrus is 8 and 26%, respectively (4). Increased risk of urinary incontinence and changes in urinary protein to creatinine (UPC) ratio in dogs are often associated with a history of sterilization but remain controversial (5). Spaying and neutering can lead to several long-term health complications that not reproductive in nature. Some of these complications include obesity, urinary incontinence, bladder stones, hypothyroidism, diabetes mellitus, hip dysplasia, ACL tears, behavioral changes (such as owner-directed aggression and anxiety), cognitive problems, and various forms of cancer (including leukemia, prostate cancer, bone cancer, skin cancer, spleen cancer, and bladder cancer) (1). To the best of our knowledge, the effects of castration and OHE on urine pH and specific gravity (USG), blood urea nitrogen (BUN) and serum creatinine (SCr) concentrations, and the UPC ratio in healthy, sexually intact adult male and female dogs have never been studied. Therefore, it remains uncertain whether sterilization may affect urinalysis results in sexually intact male and female dogs. The purpose of this was to evaluate the short-term effects of surgical sterilization on urinary parameters in healthy adult sexually intact adult male and female dogs.

2. Materials and Methods

2.1. Selection of Case

Ten healthy adult sexually intact mongrel dogs (5 males and 5 females) weighing 15 to 20 kg (6) participated in this study (Table 1). Only those aged at least 1 year were included in this study to ensure that they had reached sexual maturity (7). All dogs were kept at an NGO animal shelter in preparation for their adoption. All dogs were provided with commercial dog food (300 g per dog per day; Nutripet™; Behintash Co., Karaj, Iran) and water at all times (8). Dogs were housed in a controlled environment with a 12:12 light-dark cycle. Antiparasitic drugs (fenbendazole 150 mg, pyrantel embonate 144 mg, praziquantel 50 mg, Canivem® 0.7 mg/10 kg orally) were administered to the animals during the first two weeks of acclimatization concurrently with feeding and cleaning of the shelter. Health status was assessed using the results of a complete physical examination, complete blood count, serum chemistry, and urinalysis. If a dog's urine sample is abnormally colored or cloudy, has a pH greater than 7.5, and is confirmed to have a positive bacterial culture, the dog will be excluded. Each dog was re-examined 15 days after castration and ovariohysterectomy (9), followed by a physical examination and urinalysis. Male and female dogs underwent sterilization surgery under general anesthesia. Animals were deprived of food and water for 12 hours prior to surgery. Monitoring during surgery was at the discretion of the anesthesiologist. Dogs were premedicated with acepromazine (0.05 mg/kg, IM) and xylazine (0.5 mg/kg, IM). Ketamine (5 mg/kg, IV) and diazepam (0.25 mg/kg, IV) were used to induce anesthesia. After endotracheal intubation, general anesthesia was maintained with oxygen-vaporized isoflurane (1.2%) under intermittent positive pressure ventilation. Ketoprofen (1 mg/kg, IM) and ampicillin (20-10 mg/kg, IM) were administered during the induction phase and at the end of surgery. The study was approved according to the standards for the care and use of animals in research.

Table 1. Sex, breed, body weight, and age of 10 he

Dog	Sex	Breed	Bo
1	male	mixed	
2	male	mixed	
3	male	mixed	
4	male	mixed	
5	male	mixed	
6	female	mixed	
7	female	mixed	

2.2. Collection of Urine and Blood Samples and Analysis

Urine samples were obtained by cystocentesis and stored at 4°C until analysis by the Diagnostic Service of the Faculty of Veterinary Medicine. After centrifugation at 3000 g for 5 minutes, the urinalysis (including biochemical analysis and examination of urine sediment) was conducted within 4 hours of collection. Biomarkers of renal function included serum concentrations of Cr and BUN and urine for pH, USG, and UPC ratio. Standard serum BUN concentrations were determined by enzymatic colorimetric methods, and Cr concentrations were measured by the Jaffe method. Dipstick test results for protein concentration are negative (corresponding to the urine protein concentration of 0 g/L) , 1+ (corresponding to a urine protein concentration of 0.3 g/L). L), 2+ (corresponding to a urine protein concentration of 1 g/L), or 3+ (corresponding to a urine protein concentration of 5 g/L). The supernatant was used for the UPC ratio. Urine creatinine concentration was measured by the colorimetric method and determined by the modified Jaffè method. The USG was determined using a refractometer. An inactive sediment was defined by the absence of bacteria and less than five red blood cells, white blood cells, or epithelial cells per high-power field.

2.3. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA). The Kolmogorov-Smirnov test was used to assess the normality of the data. Data are presented as mean ± SD. for normally distributed data and range and median for nonparametric data. Depending on the data distribution, an independent samples t-test or a Mann-WhitneyU-test was used to compare the two groups. *P<0.05* was considered statistically significant for differences between groups.

3. Results

3.1. Serum Biochemistry Analysis

Serum biochemistry results such as BUN and Cr were within the laboratory reference intervals in all dogs. There was no significant difference between the mean BUN and Cr before ovariohysterectomy (16.78 ± 4.05; Figure 1A and 1.06 ± 0.11; Figure 2A, respectively) and castration (17.04 ± 3.27; Figure 1B and 1.22 ± 0.07; Figure 2B, respectively), and after ovariohysterectomy (16.90 ± 3.82; Figure 1A and 1.11 ± 0.10; Figure 2A, respectively) and castration (17.26 ± 3.39; Figure 1B and 1.24 ± 0.09; Figure 2B, respectively).

3.2. Urinalysis

All urine biochemistry and sedimentation results were within the reference intervals for all dogs. Bacterial culture results were negative for this sample.

3.3. Urine pH, USG, and UPC Ratio

The Mean UPC ratios in females and males were (0.166 ± 0.016 and 0.168 ± 0.021; Figure 3A and 0.176 ± 0.011 and 0.178 ± 0.013; Figure 3B) before and after surgical sterilization, respectively. All UPC ratios for sexually intact male and female dogs between pre- and post-neutering were not statistically significant (P>0.05). Neutering of males and females didn't effect USG and pH. Mean USG in females and males were (1.028 ± 0.005 and 1.027 ± 0.009; Figure 4A and 1.025 ± 0.005 and 1.024 ± 0.004; Figure 4B) before and after neutering, respectively. The mean pH for sexually intact bitches before and after ovariohysterectomy was (6.40 ± 0.41 and 6.60 ± 0.41; Figure 5A, respectively). In sexually intact male dogs, the mean pH was (6.30 ± 0.50 and 6.50 ± 0.35; Figure 5B) before and after castration, respectively.

4. Discussion

The present study was designed to evaluate the short-term effects of neutering on urinary and biochemical parameters in male and female dogs. The results of the UPC ratio assessment showed that the mean ratio was higher in male dogs than in female dogs before and after neutering. However, this ratio was not statistically significant in male and female dogs before and after the neutering. This finding is consistent with previous studies (10, 11). One study showed that when testing urine samples from healthy male dogs are tested with a urine dipstick, semen cancause false-positive results for protein and blood (10).

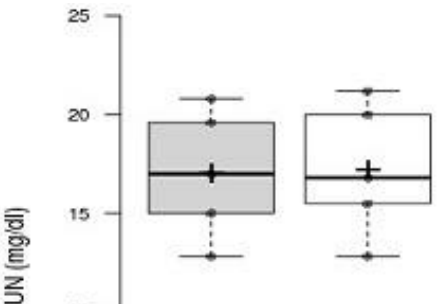

Figure 1. Box plots of blood urea nitrogen (BUN) in female dogs (A) and male dogs (B). The values of BUN are shown as a dot. The median is shown as a bold horizontal line in the center of the boxes. The mean is specified as a plus. The upper and lower limits of the values are also shown.

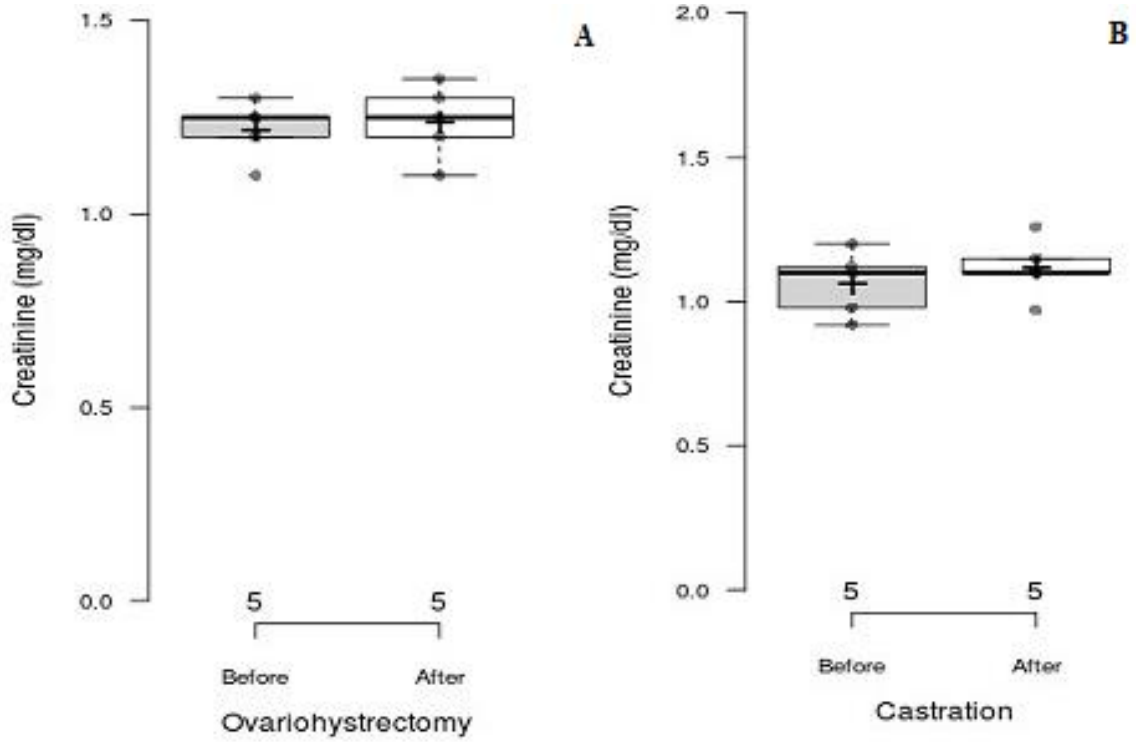

Figure 2. Box plots of serum creatinine (Cr) in female dogs (A) and male dogs (B). The leve
as a bold horizontal line in the center of the boxes. The mean is shown as a plus. The upper a

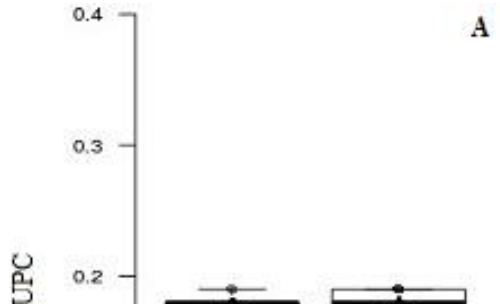

Figure 3. Box plots of urinary protein to creatinine ratio (UPC) in female dogs (A) and male dogs (B). The ratio of UPC is shown as a dot. The median is shown as a bold horizontal line in the center of the boxes. The mean is shown as a plus. The upper and lower limits of the values are also shown.

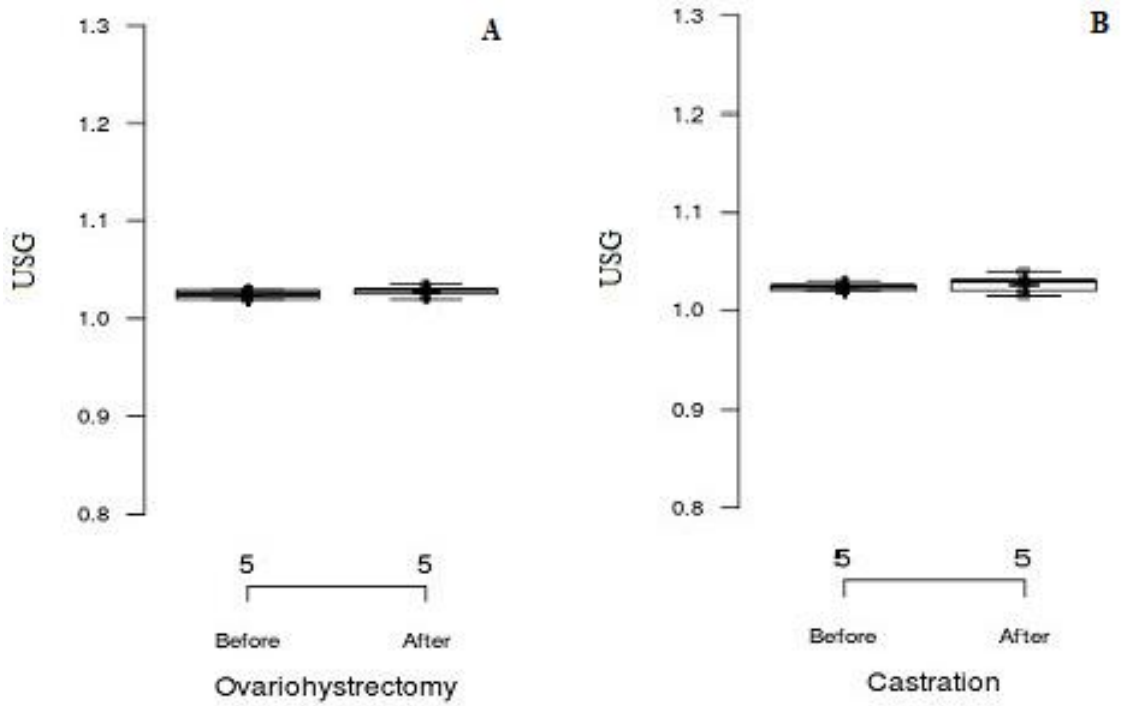

Figure 4. Box plots of urine specific gravity (USG) in female dogs (A) and male dogs (B). Th
median is shown as a bold horizontal line in the center of the boxes. The mean is shown as a p
are also shown.

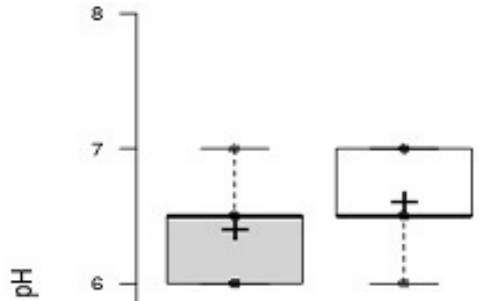

Figure 5. Box plots of urinary pH in female dogs (A) and male dogs (B). The pH values are shown as a dot. The median is shown as a bold horizontal line in the center of the boxes. The mean is shown as a plus. The upper and lower limits of the values are also shown.

Another evaluation in the same vein found that that the urine dipstick could be a viable alternative to the UPC ratio for determining proteinuria in dogs. The results indicated that if the urine test shows a small amount of protein and the USG is 1. 012 or higher, the UPC ratio should be calculated to check for proteinuria (8). In a comparison of the effect of the urine collection method on the UPC ratio in dogs, free catching was found that to have less effect on this ratio than cystosynthesis (12). The UPC ratio in urine samples collected from domestic and hospitalized dogs indicated that this ratio was higher in samples collected from domestic dogs (13). The study found that male dogs had higher mean levels of BUN and Cr. Ovariohysterectomy versus castration had less effect on BUN but more effect on serum Cr. The mean of USG in intact and spayed female dogs was lower than that of intact and spayed male dogs. Factors like age, nutrition, gender, and water content affect the interpretation of USG (14). Neutering has been observed to cause a slight increase in urine pH levels in both male and female dogs. Although the difference is not statistically significant, this information may be helpful to pet owners. It is worth noting that the effect of neutering on urine pH is quite similar to the effect of USG in both male and female dogs. Many factors can affect the acidity or alkalinity of urine. Meat consumption, as well as respiratory and metabolic acidosis, can cause symptoms such as severe diarrhea and vomiting, nausea, fever, and the need for certain substances such as ammonium chloride, high doses of ascorbic acid, citric acid, and methionine, all of which can cause urine to become more acidic. On the other hand, a meal that is low in protein and high in vegetables and grains and the consumption of bicarbonate or citrate can contribute to a more alkaline urine. In addition, certain bacteria such as *Staphylococcus aureus*, *Proteus spp*, and *Klebsiella spp*, a nd medications such as acetazolamide, sodium bicarbonate, chlorothiazide, and potassium citrate, can also lead to urinary stones. It is also important to note that urine specimen container open for a long time before testing can result in a loss of carbon dioxide, leading to a false reading of urine alkalinity (15). There has been a possible association between the long-term side effects of surgical sterilization with gonadectomy and luteinizing hormone (LH) in dogs (16). According to this, spaying and neutering is often used to prevent the birth of unwanted animals and to resolve secondary reproductive diseases. However, the effects of estrogen and testosterone on the pituitary and hypothalamus are lost when the gonads are removed. Although the primary role of LH is in reproductive function (ovulation), LH receptors are found in several normal tissues, including the thyroid and adrenal glands, the gastrointestinal tract, the cranial cruciate ligament, and lymphocytes. In addition, LH receptors (LHR) are also present in various neoplastic tissues such as lymphoma, hemangiosarcoma, mast cell tumor, transitional cell carcinoma, and osteosarcoma). The role of LHRs in normal and neoplastic non-reproductive tissues is unknown, but they may stimulate nitric oxide release and induce cell division. The exact cause of the increased incidence of multiple non-reproductive complications after castration is unknown. It may be related to the activation of the LHR in nonreproductive target tissues (16).

A significant interaction between the region and the tissue layer in the canine lower urinary tract showed that mRNA and protein expression for LHR and follicle-stimulating hormone receptor (FSHR) decreased from the bladder to the urethra in the epithelium and subepithelial stroma. Instead, it gradually increased in the muscle from the bladder to the urethra (16). The effects of neutering on bladder function in male and female dogs showed that dog neutering reduced the bladder response to muscarinic stimulation in vitro. However, they only increased the the amount of collagen in the bladder wall in females. Although an increased percentage of collagen may predispose bitches to urinary incontinence, the sex difference in this parameter suggests that there is more than one mechanism underlying the changes in the bladder response observed after neutering (18). There was no significant relationship between early spaying and urinary sphincter mechanism incontinence (USMI) in female dogs (19). There is weak evidence that the risk of urinary incontinence decreases with age at spaying**,** up to 12 months. Subsequently **,** there is no evidence of an effect of age at spaying (4, 20).Several studies have examined the association between age at ovariohysterectomy and the incidence of USMI, and some of these studies have shown an increased risk of USMI in dogs spayed before three months old or after the first heat (21). The Clinical evaluation of testosterone analogues has been proposed for the treatment of USMI in spayed female dogs (22). Dogs have LH receptors in all parts of the lower urinary tract, including the bladder and urethra. Interestingly, the number of LH receptors in the lower urinary tract of spayed female dogs with urinary incontinence is significantly higher than in unaltered females (23, 24). This new information can help veterinarians develop effective treatments for urinary incontinence in spayed female dogs and ultimately improve their quality of life (25). Urinary stones are solid particles that can form in the urinary tracts and are composed mainly of mineral salts. Banfield Pet Hospital's analysis of more than two million dogs shows that spayed and neutered dogs have a higher risk of developing urinary stones, including urinary crystals, kidney stones, and bladder stones, than unaltered dogs. This information can help pet owners take preventive measures to ensure the health of their pets' urinary systems (26). The study found castration and ovariohysterectomy had no short-term effect on urinary parameters in healthy adult sexually intact male and female dogs. However, it is uncertain how sterilization affects urinalysis results in sexually intact dogs, and more research with larger sample sizes is needed to determine the impact.

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Authors' Contribution
Study concept and design: M.A.H., and M.Y.G
Data acquisition: M.A.H., H.R.M., and M.Y.G
Data analysis and interpretation of data: M.A.H.
Drafting of the manuscript: M.A.H., and H.R.M.
Critical revision of the manuscript for important intellectual content: M.A.H., and H.R.M.
Statistical analysis: M.A.H.
Administrative, technical, and material support: M.A.H., H.R.M., and M.Y.G

Ethics
The animal experimental protocol was approved by the Animal Research Ethical Committee for Scientific Researches of Semnan University (IR.SU.REC.1399.6).

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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