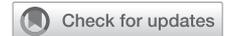


## BASIC SCIENCE

# Intracavernous Injection of Autologous Platelet-Rich Plasma Ameliorates Hyperlipidemia-Associated Erectile Dysfunction in a Rat Model



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## ABSTRACT

**Introduction:** Hyperlipidemia is associated with an increased risk of erectile dysfunction (ED) mediated by endothelial damage. Platelet-rich plasma (PRP) contains numerous angiogenic growth factors. Currently, evidence supporting the use of PRP for ED treatment is limited.

**Aim:** We investigated PRP in a rat model of hyperlipidemia-associated ED.

**Methods:** Thirty 2-month-old male Sprague–Dawley rats were randomly divided into 3 groups. 20 rats were fed a high-fat diet for 5 months and were randomly divided into 2 groups: (i) rats in the H group received supernatant injection into the corpus cavernosum weekly for 4 weeks; (ii) rats in the H + PRP group received PRP injection into the corpus cavernosum weekly for 4 weeks. 10 rats were fed a standard diet for 5 months and received supernatant injection into the corpus cavernosum weekly for 4 weeks (N group). 7 days after the 4th injection, all rats underwent erectile function testing and then euthanasia.

**Main outcome measures:** Erectile function was evaluated by measuring intracavernous pressure (ICP) and mean arterial pressure (MAP). Serum and penile tissue were collected for metabolic variable assessment and histochemical examination, respectively.

**Results:** Intracavernous pressure/MAP and area under the curve/MAP ratios were significantly higher in the N and H + PRP groups than in the H group. Insulin-like growth factor-1, brain-derived neurotrophic factor, and vascular endothelial growth factor levels were significantly higher in the H + PRP group than in the N and H groups. Corporal neuronal nitric oxide synthase, endothelial nitric oxide synthase, and endothelial cells were weakly expressed in the H group compared with the N and H + PRP groups. Intracorporal oxidative stress and apoptotic index were significantly higher in the H group than in the N and H + PRP groups.

**Conclusions:** This preclinical evidence suggests that clinical trials of PRP in men with ED should be considered. PRP may play a role in ED management. **Huang YC, Wu CT, Chen MF, et al. Intracavernous Injection of Autologous Platelet-Rich Plasma Ameliorates Hyperlipidemia-Associated Erectile Dysfunction in a Rat Model. Sex Med 2021;9:100317.**

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**Key Words:** Platelet rich plasma; Hyperlipidemia; Fat; Diet; Erectile Dysfunction

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## INTRODUCTION

Erectile dysfunction (ED) is common disorder and exerts a substantial negative influence on quality of life for both the affected man and his sexual partner(s).<sup>1</sup> ED is estimated to affect between 16%–22% of men depending on the definition utilized and the population studied.<sup>2</sup> The prevalence of ED increases with age.<sup>2</sup>

Effective treatment of ED typically begins with the modification of risk factors and oral pharmacotherapy with phosphodiesterase type 5 inhibitors (PDE5Is).<sup>3</sup> However, PDE5Is may be ineffective in 30–40% of the population and fail to resolve the underlying disorders that cause ED.<sup>4</sup> In men who show no response to or who cannot tolerate PDE5Is, more invasive medical therapies (eg, vacuum erection devices, intraurethral alprostadil, and intracavernous injections) and surgical penile prosthesis implantation may be considered.<sup>5</sup> Patient compliance and tolerance with these therapies is variable. A treatment that reverses or cures the underlying physiological insult of ED is preferable to currently available “symptomatic relief” therapies.

Hyperlipidemia is a prevalent, chronic condition that affects penile hemodynamics; it is an independent risk factor for ED.<sup>6,7</sup> Angiogenic growth factors (eg, vascular endothelial growth factor [VEGF] and insulin-like growth factor-1 [IGF-1]), are present in corporal tissue. These proteins tend to induce proliferation and angiogenesis and may protect against vascular ED.<sup>8</sup> It is reasonable to hypothesize that vasculature (including penile vasculature) function is improved through delivery of VEGF and IGF-1 to penile tissue.

Nikolidakis *et al* demonstrated that platelets outside the blood stream release proliferative growth factors, including VEGF and IGF-1.<sup>9</sup> The use of platelet-rich plasma (PRP) in orthopedic medicine is relatively well established; however, to date, little robust evidence has been provided to support the efficacy of PRP for ED.<sup>10,11</sup> Autologous PRP is therefore a promising but unproven therapy for hyperlipidemia-associated ED and should be implemented only in appropriately designed, IRB-approved clinical trials.<sup>5</sup>

In this study, we investigated the efficacy of autologous PRP for treatment of impaired penile hemodynamics in a rat model of hyperlipidemia-associated ED. We hypothesized the following: (i) rats fed a high-fat diet exhibit impaired penile hemodynamics and histological changes and (ii) treatment with autologous PRP at least partially mitigates functional and histological changes in the penis associated with high-fat diets.

## METHODS

### Experimental Animal Groups and Design

Thirty healthy male Sprague–Dawley rats (2 months old, 320–370 g) were obtained from BioLASCO Taiwan Co, Ltd All animal care, treatments, and surgical procedures followed the rules of the Association for Assessment and Accreditation of

Laboratory Animal Care International and were approved by the Institutional Animal Care and Use Committee of the Laboratory Animal Center at our institution. The institutional review board of our university approved this research.

Rats were randomly divided into 3 groups. 10 rats were fed a diet of standard chow consisting of 13% fat for 5 months; this group served as the negative controls (N group). The remaining 20 rats were fed a high-fat diet consisting of 48% fat (Research Diets, New Brunswick, NJ, USA) for 5 months. This protocol has been demonstrated to induce impaired penile hemodynamics in previous studies.<sup>6,7</sup>

After rats were fed the high-fat diet for 5 months, blood was obtained from the tail vein of all rats for PRP preparation (see details below). Whole blood was subjected to centrifugation for isolation of the platelet-rich portion. The 20 hyperlipidemic rats were randomly divided into 2 groups: (i) the H group consisted of rats ( $n = 10$ ) that received a weekly injection of supernatant collected from the second centrifugation into the corpus cavernosum for 4 weeks and (ii) the H + PRP group consisted of rats ( $n = 10$ ) that received a weekly injection of PRP into the corpus cavernosum for 4 weeks. Negative control animals received a weekly supernatant injection into the corpus cavernosum for 4 weeks.

7 days after the 4th injection, all rats underwent penile hemodynamic testing. After penile hemodynamic testing, blood and penile tissue were obtained for biochemical analysis and molecular studies. Animals were then euthanized through bilateral thoracotomy.

### Preparation of PRP

Whole blood of 30 rats was drawn through the tail vein for preparation of PRP and was transferred to siliconized tubes containing the anticoagulant citrate dextrose solution A (ACD-A, BD, Franklin Lakes, NJ, USA). Blood was centrifuged at 400 g for 30 minutes; the supernatant, which contained the PRP, was transferred to another tube for a second centrifugation at 1,500 g for 15 minutes. The top layer obtained from the second centrifugation was aspirated, and the remaining layer was collected and diluted with the supernatant until the platelet concentration was  $1.5 \times 10^6$  platelet/ $\mu\text{L}$ . PRP was stimulated with 175  $\mu\text{g}/\text{mL}$  chitosan and stored at  $-20^\circ\text{C}$  until further use.<sup>12</sup>

### Determination of Growth Factor Concentrations

PRP samples at a concentration of  $1.5 \times 10^6$  platelets/ $\mu\text{L}$  were analyzed for angiogenic growth factors, including IGF-1, brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), and VEGF, by using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Merck KGaA, Darmstadt, Germany). The supernatant from the second centrifugation was tested for the same cytokines by using the same assays. Absorbance was measured at 450 nm on a

**Table 1.** Body weight and metabolic variables in experimental animals

	N	H	H + PRP	P Value
Body weight (grams)	576 ± 12.0	673 ± 21.7*	671 ± 25.8*	<b>.0030</b>
Total cholesterol (mg/dL)	70.6 ± 3.38	70.3 ± 3.12	71.1 ± 4.36	.9870
Triglyceride (mg/dL)	104 ± 18.2	101 ± 43.6	127 ± 10.3	.7335
LDL (mg/dL)	8.9 ± 0.59	12.6 ± 0.71*	12.6 ± 0.91*	<b>.0018</b>
HDL (mg/dL)	28.3 ± 1.23	23.3 ± 0.99*	22.2 ± 1.35*	<b>.0033</b>
Glucose (mg/dL)	297 ± 12.2	376 ± 21.3	381 ± 26.2*	<b>.0337</b>
Testosterone (ng/mL)	1.3 ± 0.11	1.2 ± 0.15	1.5 ± 0.10	.2974
Nitric oxide (μM)	6.7 ± 0.68 <sup>†</sup>	2.5 ± 0.47	7.0 ± 1.68 <sup>†</sup>	<b>.0121</b>

H = high-fat diet; HDL = high-density lipoprotein; LDL = low-density lipoprotein; N = normal diet; PRP = platelet-rich plasma.

Ten rats were selected for analysis from each group.

Bold *P* values indicate statistical significance.

\*Versus N group *P* < .05.

<sup>†</sup>Versus H group *P* < .05.

spectrophotometric reader. Growth factor concentration was calculated based on a standard curve and is expressed as pg/mL.

### PRP Injection

For surgical procedures, all rats were anesthetized with Zoletil 50 (20 mg/kg). A lower abdominal midline incision was made to expose the penis. The penile base was temporarily ligated with a polyethylene-90 tube. Each rat received an injection of 200 μL autologous supernatant (N and H groups) or 200 μL autologous activated PRP (containing  $3 \times 10^8$  platelets) into the corpus cavernosum (H + PRP group).<sup>12</sup> Following the injection, the needle was left in place for 5 minutes to allow diffusion of the injected materials. After treatment, the wound was closed in one layer with absorbable sutures. All rats received injections once weekly for 4 contiguous weeks.

### Erectile Function Measurement

At 7 days after the 4th injection, penile hemodynamics was investigated according to our standard protocol.<sup>13–15</sup> Under anesthesia with Zoletil 50 (20 mg/kg), the bilateral cavernous nerves and penile crus were exposed and isolated through a lower midline laparotomy. A 23-gauge needle filled with heparinized saline (200 U/mL) was used to cannulate the penile crus and monitor intracavernous pressure (ICP). A polyethylene-50 tube filled with heparinized saline (200 U/mL) was inserted into the left common carotid artery and connected to a pressure transducer to continuously monitor mean arterial pressure (MAP). The cavernous nerves were then stimulated with a bipolar electrode connected to a stimulator at 20 Hz and 1.5 mAmp (A-M Systems, Sequim, WA, USA) for 50 seconds. Stimulation was conducted 3 times on each side; a resting period of 2 minutes was observed between stimulations to allow nerve recovery. The maximum increase in ICP from each side in each animal was determined for further analysis. To normalize for variation in systemic blood pressure, penile hemodynamics was reported as

the ratios of ICP to MAP and the area under the curve to MAP during the entire erectile response.

### Griess Nitrite Assay

To measure the levels of serum nitrite (a stable and nonvolatile breakdown product of nitric oxide [NO]), a Griess nitrite assay was conducted according to the standard protocol. Briefly, 50 μL of nitrite standard or serum from subject rats was incubated with 50 μL of the Griess reagent for 5–10 minutes. N-1-naphthylethylenediamine dihydrochloride solution was added and incubation continued for another 5–10 minutes (Promega, WI, USA). Absorbance was measured at 550 nm on the spectrophotometric reader. Nitrite content was determined based on a standard curve and is expressed as μM.<sup>14</sup>

### Immunohistochemical Staining

Freshly dissected penile tissues were harvested, fixed, and processed for immunohistochemical staining, as previously described.<sup>6,7,13–15</sup> After fixation penile tissues were embedded in OCT Compound (American Master Tech Scientific, Lodi, CA, USA), and 5-μm thick section were cut and mounted on glass slides. The sections were then processed for immunohistochemical staining with mouse antineuronal nitric oxide synthase (nNOS, 1:100; BD Biosciences, San Jose, CA, USA), mouse anti-rat endothelial cell antigen-1 (1:400, RECA-1; Abcam, Cambridge, MA, USA), phalloidin (1:400; Invitrogen, Carlsbad, CA, USA), and mouse anti-8-hydroxy-2'-deoxyguanosine (8-OHdG, 1:50, JaICA, Nikken Seil Co, Japan) by using standard techniques. Cell apoptosis was assessed using a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL, Roche Diagnostics Corporation, Indianapolis, IN, USA) kit. Briefly, the slides were incubated in methanol/hydrogen peroxide for 5 minutes to quench endogenous peroxidase activity. Subsequently, the specimens were incubated in TUNEL reagent (1 hour at 37°C) and anti-digoxigenin-peroxidase (30 minutes at room temperature).

**Table 2.** Concentration of growth factors in supernatant and PRP

	Supernatant*	PRP	<i>P</i> Value
Platelet ( $10^3/\mu\text{L}$ )	$<5^\dagger$	$1,500^\ddagger$	
IGF-1 (pg/mL)	$211 \pm 77.8$	$92,735 \pm 15,990$	<b>.0006</b>
BDNF (pg/mL)	$1.0 \pm 0.53$	$2,558 \pm 235$	<b>.0001</b>
bFGF (pg/mL)	$2.0 \pm 1.28$	$4.0 \pm 1.51$	.3270
VEGF (pg/mL)	$0.4 \pm 0.09$	$2.0 \pm 0.24$	<b>.0002</b>

BDNF = brain-derived neurotrophic factor; bFGF = basic fibroblast growth factor; IGF-1 = insulin-like growth factor-1; PRP = platelet-rich plasma; VEGF = vascular endothelial growth factor.

6 and 10 rats were selected for analysis from the supernatant and platelet-rich plasma groups, respectively.

Bold *P* values indicate statistical significance.

\*The supernatant was collected from the second centrifugation of platelet-rich plasma preparation.

<sup>†</sup>Minimal detectable value of platelet concentration was  $5 \times 10^3/\mu\text{L}$ .

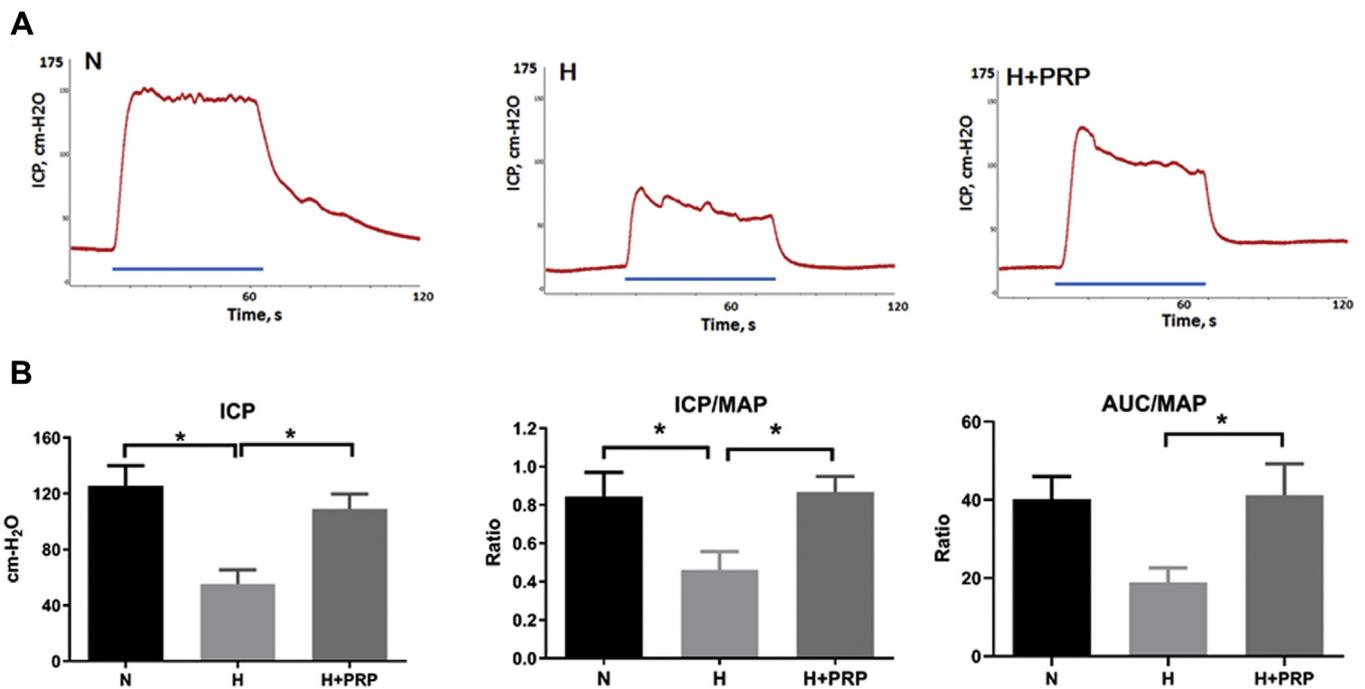
<sup>‡</sup>The pellet from the second centrifugation was collected and diluted with supernatant until the platelet concentration was  $1.5 \times 10^6$  platelet/ $\mu\text{L}$ .

Finally, nuclear staining was performed with 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, Carlsbad, CA, USA), followed by counterstaining with fluorescence mounting medium.

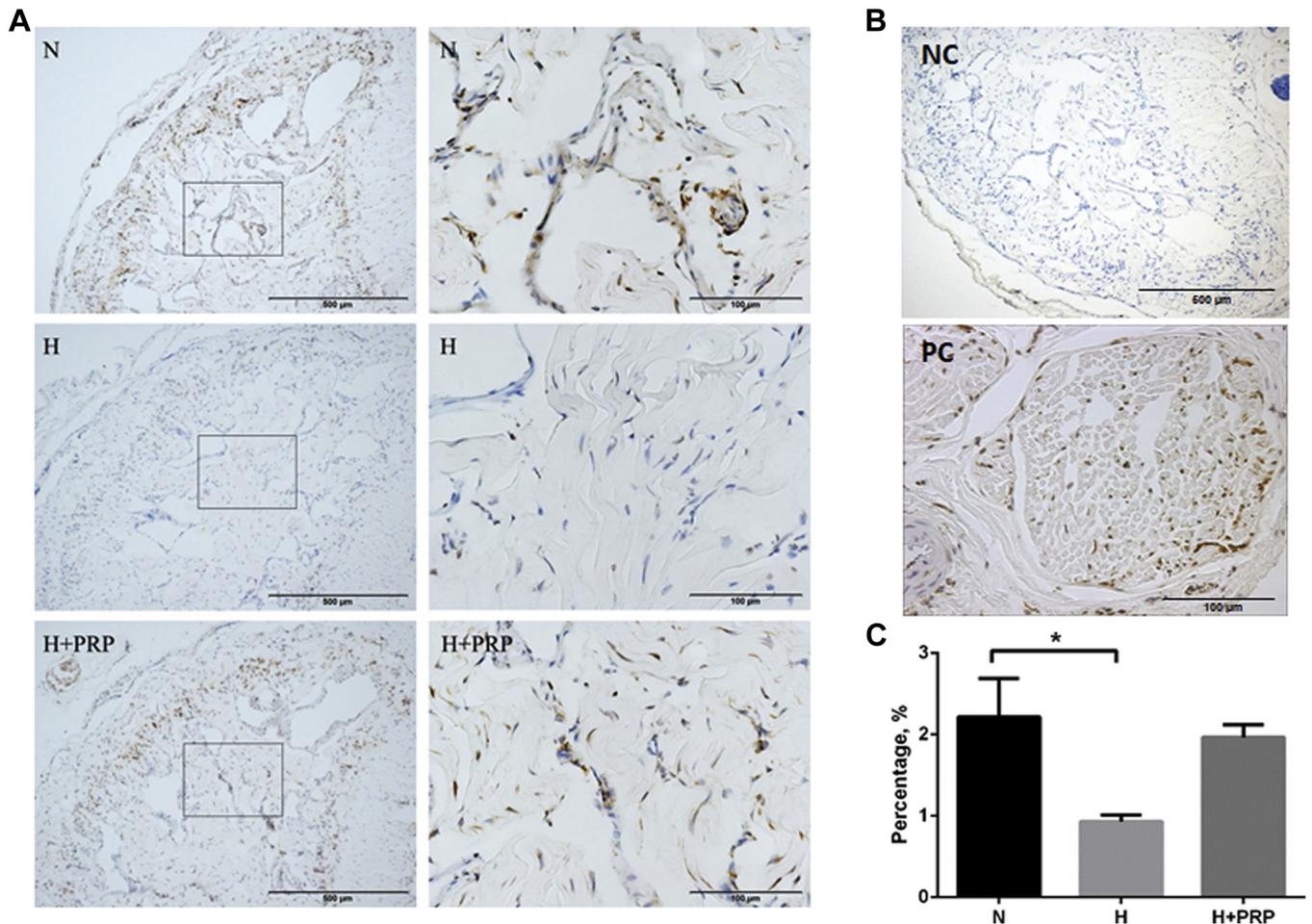
### Western Blot Analysis

Primary antibodies used in the experiment were mouse anti-endothelial nitric oxide synthase (eNOS, 1:1,000; BD Biosciences, San Jose, CA, USA) and mouse anti- $\beta$ -actin antibody (1:10,000; Santa Cruz, Dallas, Texas, USA). Western blot analysis was performed as previously described.<sup>13,14</sup> Briefly, the urethra was

dissected from the penile tissue. Approximately 50 mg of corporal tissue from the penile base were collected and homogenized in 1 mL of tissue protein extraction exigent (Thermo Fisher Scientific Inc, Waltham, MA, USA). The lysate was centrifuged at 17,900 g for 15 minutes, and the supernatant was collected as the protein samples. An equal amount of protein (50  $\mu\text{g}$ ) from each tissue was electrophoresed in 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a polyvinylidene fluoride membrane (Merck, Darmstadt, Germany), and incubated with primary antibody. Protein detection was performed using an ECL kit (Amersham Life Sciences, Pittsburgh, PA, USA). The resulting images were analyzed through densitometry.



**Figure 1.** Platelet-rich plasma (PRP) improved erectile function upon cavernous nerve electrostimulation. (A) Representative tracings of intracavernous pressure (ICP) for the N, H, and H + PRP groups. (B) Erectile function is presented as ICP, ICP/mean arterial pressure (MAP), and the area under the curve (AUC)/MAP ratios in each group. The blue bar represents cavernous nerve electrostimulation for 50 seconds. \**P* < .05.



**Figure 2.** Neuronal nitric oxide synthase (nNOS) staining of the corpus cavernosum. (A) Representative images of nNOS-positive fibers stained with nNOS antibody (brown) for the N, H, and H + PRP groups. The boxed areas in the left panels indicated the areas selected for amplification in the right panels. Left and right scale bars represent 500  $\mu\text{m}$  and 100  $\mu\text{m}$ , respectively. (B) Negative control (NC) was stained without primary antibody. The dorsal penile nerve was used as a positive control (PC). The top and bottom scale bars represent 500 and 100  $\mu\text{m}$ , respectively. (C) nNOS content as a percentage of total intratunical area. The H group had significantly fewer nNOS-positive fibers than the N group. After PRP treatment, the H + PRP group had higher mean nNOS staining in the corpus cavernosum, but the difference was not significant. \* $P < .05$ .

### Image and Statistical Analyses

For histological image analysis of nNOS, RECA-1, phalloidin and 8-OHdG, the entire corpus cavernosum was imaged using a digital camera (Nikon DXM1200) coupled to ACT-1 software (Nikon Instruments Inc, Melville, NY, USA). Image quantification was performed with Image-Pro plus imaging software (Media Cybernetics, Silver Spring, MD, USA). The results are expressed as the percentage of area positive for antibody divided by the total area of the entire corpus cavernosum. For TUNEL staining, the results are expressed as the percentage of all TUNEL-positive nuclei counted in the entire corpus cavernosum versus the total nuclei in the entire corpus cavernosum.

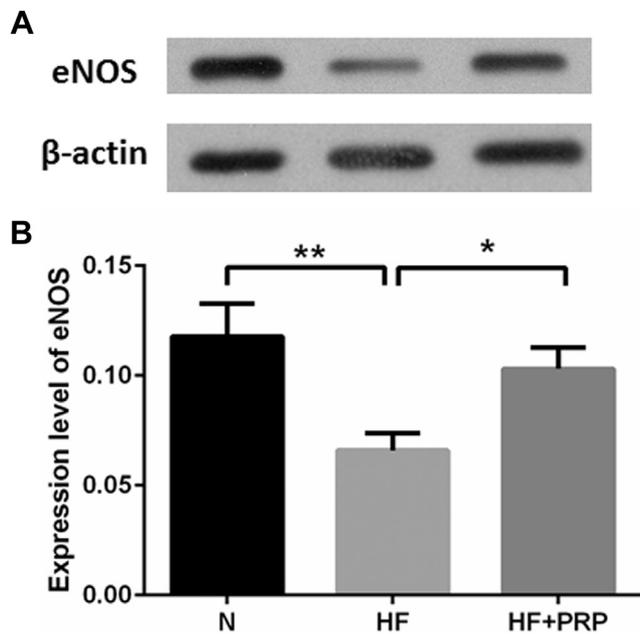
Numerical data were analyzed using Prism version 9 (GraphPad Software, La Jolla, CA, USA) and are expressed as mean  $\pm$  standard error of the mean. Comparisons between 2 groups were made using the 2-tailed  $t$  test. Comparisons between multiple groups were made using one-way analysis of variance

followed by the Tukey–Kramer test for post hoc comparisons. A  $P$  value of  $<.05$  was considered statistically significant.

## RESULTS

### Body Weight and Metabolic Variables

Body weight and metabolic variables are reported in Table 1. Mean body weight was significantly higher in the H and H + PRP groups than in the N group ( $P = .003$ ). Serum low-density lipoprotein and glucose levels were significantly higher in the H and H + PRP groups than in the N group ( $P = .0018$  and  $.0337$ , respectively). Serum high-density lipoprotein was significantly lower in the H and H + PRP group than in the N group ( $P = .0033$ ). The N and H + PRP groups had significantly higher serum NO levels than the H group ( $P = .0121$ ). No significant differences in total cholesterol, triglyceride, and testosterone levels were observed between groups.



**Figure 3.** Western blotting for endothelial nitric oxide synthase (eNOS). (A) eNOS normalized to  $\beta$ -actin. (B) Mean eNOS positivity. The H group had significantly lower eNOS than the N and H + PRP groups. \* $P < .05$ ; \*\* $P < .01$ .

### Concentrations of Growth Factors Released from PRP

Platelets were undetectable in the supernatant collected from the second centrifugation compared with PRP ( $<5 \times 10^3$  vs  $1,500 \times 10^3$  platelet/ $\mu$ L, Table 2). Mean levels of IGF-1, BDNF, and VEGF were significantly higher in the PRP than in the supernatant (all  $P < .05$ ). PRP had higher mean bFGF levels than the supernatant, but the difference did not reach statistical significance ( $P = .3270$ ).

### Penile Hemodynamic Study

Representative images of ICP testing are shown in Figure 1. Mean ICP was significantly higher in the N ( $126 \pm 14.4$  cm H<sub>2</sub>O) and H + PRP ( $109 \pm 10.8$  cm H<sub>2</sub>O) groups than in the H group ( $55 \pm 10.1$  cm H<sub>2</sub>O,  $P = .0009$ ). The ratios of ICP/MAP were  $0.84 \pm 0.127$ ,  $0.87 \pm 0.082$ , and  $0.46 \pm 0.096$  in the N, H + PRP, and H groups, respectively ( $P = .0155$ ). Similarly, area under the curve /MAP ratios in the N ( $40 \pm 5.8$ ) and H + PRP ( $41 \pm 7.9$ ) groups were significantly higher than in the H ( $19 \pm 3.6$ ) group ( $P = .0338$ ). However, no statistically significant difference in MAP was observed between the groups ( $P = .8408$ ).

### nNOS Positivity in the Corpus Cavernosum

Representative images of nNOS staining in the corpus cavernosum are shown in Figure 2. The H group ( $0.9\% \pm 0.08\%$ ) had significantly lower mean nNOS staining in the corpus cavernosum than the N group ( $2.2\% \pm 0.47\%$ ,  $P = .0199$ ). Compared with the H group ( $0.9\% \pm 0.08\%$ ), the H + PRP group ( $2.0\% \pm 0.16\%$ ) had higher mean nNOS staining in the

corpus cavernosum, but the difference was not significant ( $P = .0779$ ).

### eNOS Western Blotting in the Corpus Cavernosum

Representative images of Western Blotting are shown in Figure 3. Cavernous eNOS protein expression was significantly decreased in the H group ( $0.06 \pm 0.008$ ) compared with the N group ( $0.12 \pm 0.015$ ,  $P = .0059$ ). The H + PRP group exhibited significantly increased eNOS protein expression ( $0.10 \pm 0.01$ ) compared with the H group ( $P = .0411$ ).

### Endothelial Content in the Corpus Cavernosum

Representative images of RECA-1 staining in the corpus cavernosum are shown in Figure 4. The H group ( $4.8\% \pm 0.28\%$ ) had significantly lower mean RECA-1 staining in the corpus cavernosum than the N group ( $6.9\% \pm 0.35\%$ ,  $P = .0008$ ). Compared with the H group ( $4.8\% \pm 0.28\%$ ), the H + PRP group ( $6.1\% \pm 0.37\%$ ) had significantly higher mean RECA-1 staining in the corpus cavernosum ( $P = .0406$ ).

### Smooth Muscle Content in the Corpus Cavernosum

No significant difference was observed in phalloidin staining among the 3 groups ( $P = .9163$ , data not shown).

### Oxidative Stress in the Corpus Cavernosum

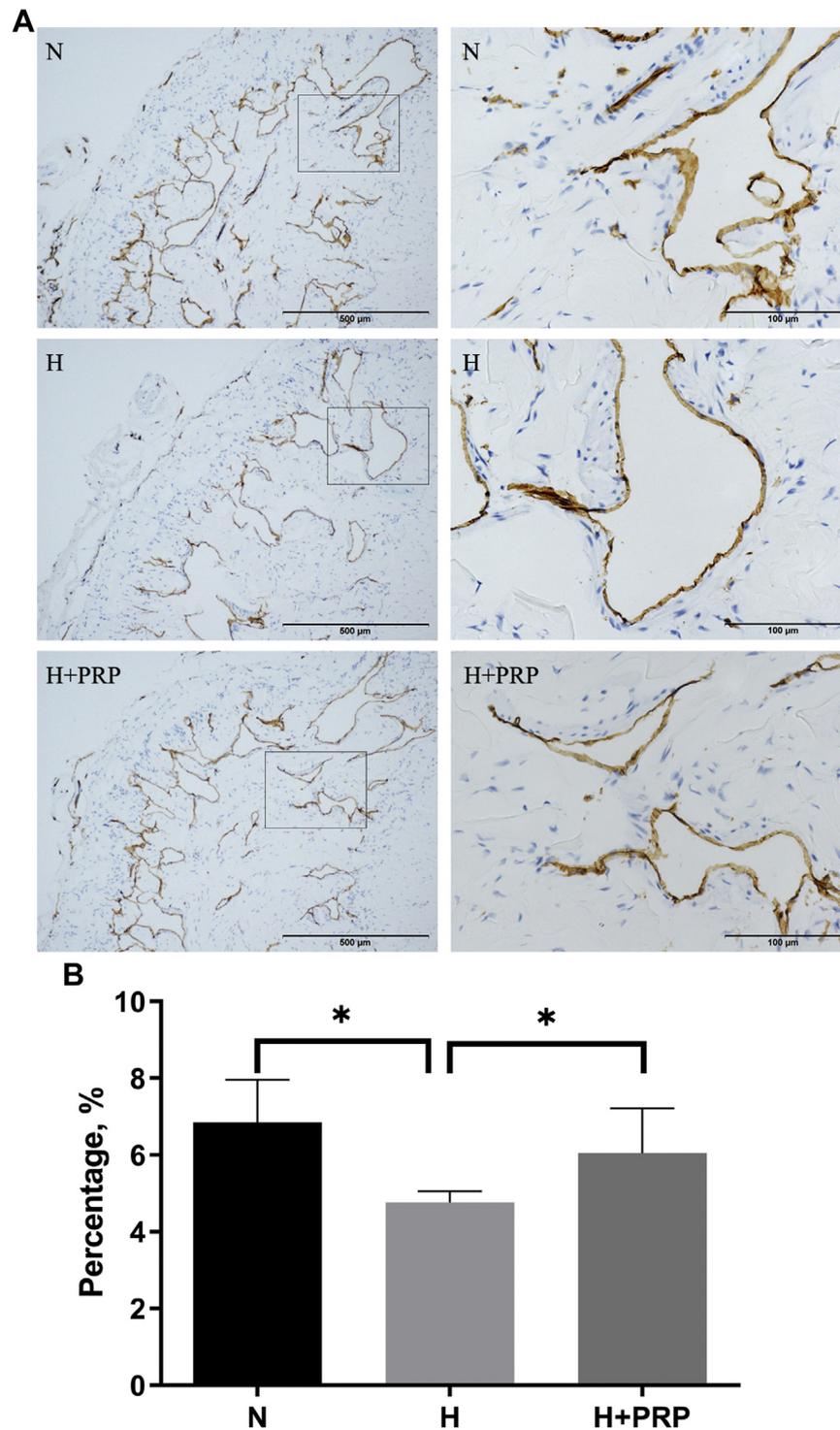
Representative images of 8-OHdG staining in the corpus cavernosum are shown in Figure 5. Mean 8-OHdG positivity was markedly higher in the H group ( $1.07\% \pm 0.176\%$ ) than in the N group ( $0.3\% \pm 0.055\%$ ,  $P < .0001$ ). Mean 8-OHdG positivity was significantly lower in the H + PRP group ( $0.3\% \pm 0.037\%$ ) than in the H group ( $P < .0001$ ).

### Apoptosis Analysis in the Corpus Cavernosum

Representative images of TUNEL staining in the corpus cavernosum are shown in Figure 6. Mean TUNEL positivity was significantly higher in the H group ( $1.6\% \pm 0.17\%$ ) than in the N group ( $0.35\% \pm 0.032\%$ ,  $P < .0001$ ). Mean TUNEL positivity was significantly lower in the H + PRP group ( $0.72\% \pm 0.123\%$ ) than in the H group ( $P < .0001$ ).

## DISCUSSION

Despite the widespread commercialization of PRP for ED management, there is at this time little evidence to support its use for this indication.<sup>11</sup> In this study, a rat model of hyperlipidemia-associated ED was used to investigate the therapeutic effects of PRP. Rats fed a high-fat diet demonstrated numerous abnormalities in both neural and endothelial tissues responsible for erectile function.<sup>16</sup> Hyperlipidemic rats treated with intracavernous injection of PRP exhibited substantially superior penile ultrastructural and hemodynamic parameters compared with untreated hyperlipidemic rats. These encouraging data

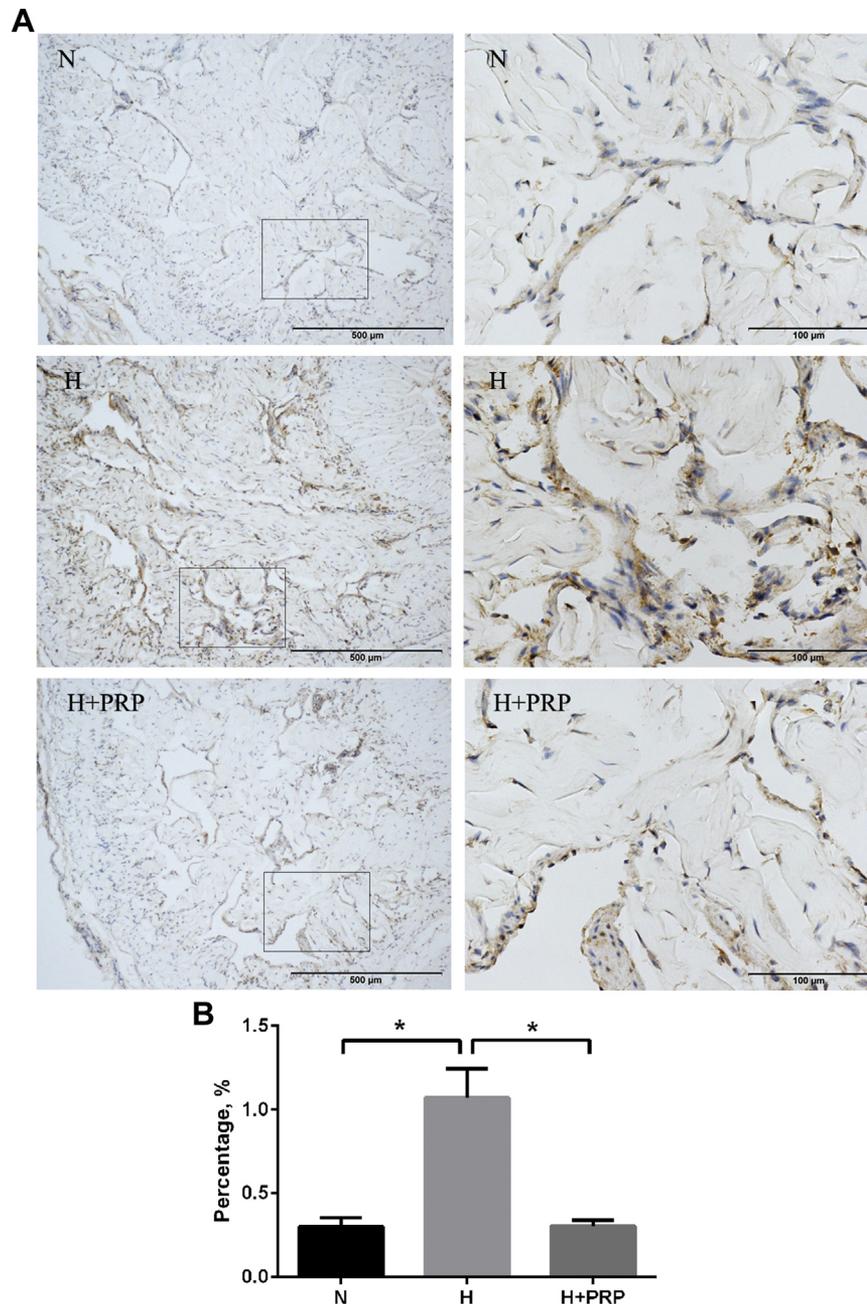


**Figure 4.** Endothelial content in the corpus cavernosum. (A) Representative images showing staining with mouse anti-rat endothelial cell antigen-1 (RECA-1) antibody (brown) for the N, H, and H + PRP groups. The boxed areas in the left panels indicate the areas selected for amplification in the right panels. Left and right scale bars represent 500 and 100  $\mu\text{m}$ , respectively. (B) RECA-1 content as a percentage of total intratunical area. The H group had significantly lower RECA-1 than the N and H + PRP groups.  $*P < .05$ .

suggest mechanisms by which PRP may be beneficial in men with hyperlipidemia-associated ED.<sup>17</sup>

PRP has been identified as an effective strategy for tissue regeneration; however, the safety of PRP in tumorigenicity and

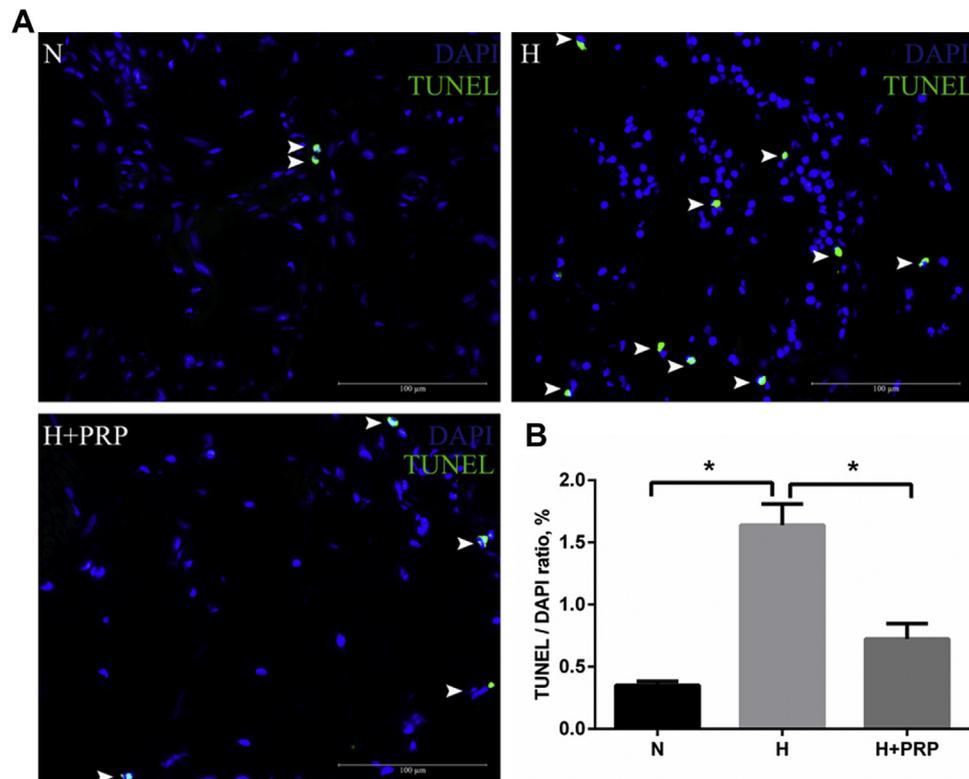
relapse has not been resolved.<sup>18</sup> Secretory proteins contained in the  $\alpha$ -granules of platelets include platelet-derived growth factor, transforming growth factor  $\beta$ -1, and VEGF, which are able to stimulate angiogenesis, induce tumor lymphangiogenesis, and



**Figure 5.** Analysis of oxidative stress through 8-hydroxy-2'-deoxyguanosine (8-OHdG) staining of the corpus cavernosum. (A) Representative images showing staining with 8-OHdG (a product of oxidatively damaged DNA) antibody (brown) for the N, H, and H + PRP groups. The boxed areas in the left panels indicated the areas selected for amplification in the right panels. Left and right scale bars represent 500  $\mu\text{m}$  and 100  $\mu\text{m}$ , respectively. (B) 8-OHdG content as a percentage of total intratunical area. The H group had significantly higher 8-OHdG than the N and H + PRP groups. \* $P < .0001$ .

enhance the nodal metastasis rate.<sup>19,20</sup> However, most related studies have focused on nerve regeneration and have used cavernous nerve injury as a model for postprostatectomy ED, and the primary indication for radical prostatectomy in humans is cancer.<sup>12,21,22</sup> In this study, PRP was injected at the site of the corpus cavernosum to recover erectile function in a rat model of hyperlipidemia-associated ED; this approach is more reliable for translating the findings from animals to humans.

Platelet products for therapeutic administration are classified into one of 4 categories based on their leucocyte and fibrin contents: (i) pure platelet-rich plasma (P-PRP); (ii) leukocyte- and platelet-rich plasma (L-PRP); (iii) pure platelet-rich fibrin; and (iv) leukocyte- and platelet-rich fibrin.<sup>23</sup> Classical manual PRP preparation entails a 2-step centrifugation procedure performed using a pipette, with no objective quantification of platelet content.<sup>20</sup> A strength of our study is that we quantified



**Figure 6.** Analysis of apoptosis through terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining of the corpus cavernosum. (A) Representative images of apoptosis-positive cells stained with Alexa Fluor 488 TUNEL (green) for the N, H, and H + PRP groups. The scale bar represents 100  $\mu$ m. (B) TUNEL-positive nuclei as a percentage of total intratunical nuclei. The H group had significantly more apoptosis-positive cells than the N and H + PRP groups. \* $P < .0001$ .

the platelet content of our PRP product; therefore, we can conclude that observed results were mediated purely by platelets and not by other blood component contaminants.

Administration of cytokines has shown to exert positive effects on erectile function in animal models of cavernous nerve injury and vascular-mediated ED.<sup>8</sup> Protein delivery may be accomplished through the following: (i) administration of actual proteins; (ii) administration of genes through various vectors; and (iii) administration of cells that produce proteins of interest.<sup>24</sup> A notable disadvantage of direct growth factor delivery is that many proteins are associated with inflammatory response.<sup>24</sup> Gene therapy is limited by issues with delivery and activation of transferred genetic material. Cell-based therapy may minimize these limitations; furthermore, cells contain a multitude of growth and differentiation factors that may lead to synergistic restorative effects.

PRP may be the optimal cell type because of the ease of harvesting autologous cells. Platelets play an important role in coagulation and promotion of wound healing following injury; these specialized cells contain key growth factors responsible for regenerative functions, including stimulating cell proliferation, matrix remodeling, inducing angiogenesis, recruiting stem cells, and modulating inflammatory responses.<sup>20</sup>

Although PRP contains numerous growth factors with potentially beneficial effects on erectile function, most studies of

growth factors in animal models of ED have focused on IGF-1, BDNF, bFGF, and VEGF.<sup>8</sup> These cytokines have been shown to ameliorate impaired penile hemodynamics in animal models of ED.<sup>25,26</sup> We selectively measured the concentrations of IGF-1, BDNF, bFGF, and VEGF in our PRP product and found that it was rich in these trophic factors. Additional studies to verify the molecular mechanisms underlying the relationship among PRP, cytokines, and ED are warranted.

Oxidative injury may be correlated with DNA oxidation, protein oxidation, lipid peroxidation, and decreased synthesis and bioavailability of NO.<sup>27</sup> IGF-1 suppresses apoptosis and enhances the paracrine function of muscle-derived stem cells under oxidative stress.<sup>28</sup> BDNF-induced mitophagy plays a protective role against endothelial cell damage in hyperglycemic conditions.<sup>29</sup> VEGF promotes signaling through an alternate VEGF receptor-2 in the setting of mitochondrial dysfunction and oxidative stress.<sup>30</sup> From this previous study, it is logical to conclude that the beneficial histological and functional results of PRP treatment are derived from the PRP-mediated delivery of IGF-1, BDNF, and VEGF.

We did not include a treatment arm of healthy rats that were given PRP. Numerous inflammatory markers potentially relevant to both ED and hyperlipidemia (eg, interleukin [IL]-6, IL-8, IL-18, and C-reactive protein) as well as the phospho-eNOS

expression in the corpus cavernosum were not investigated. The safety of PRP in terms of tumorigenicity is unclear.<sup>18</sup> Secretory proteins contained in the  $\alpha$ -granules of platelets include platelet-derived growth factor, transforming growth factor  $\beta$ -1, and VEGF, all of which have been associated with angiogenesis (including in the context of tumors) and enhancement of the nodal metastasis rate.<sup>19,20</sup> Extrapolation of these results to the human condition of ED requires further investigation. Further research is required to investigate the optimal dosage, treatment interval, safety, and long-term durability of PRP for ED treatment. The application of PRP in the clinical context presents significant limitations and challenges. Nevertheless, this preliminary work suggests that further research on the use of PRP for ED treatment is warranted. Such work should be conducted in the context of institutional review board approved studies, conducted at no or minimal expense to participating men.<sup>5</sup>

## CONCLUSIONS

Rats fed a high-fat diet developed hyperlipidemia and impaired penile hemodynamics. Intracavernous injections of autologous PRP partially reversed the histological and hemodynamic consequences of hyperlipidemia in the penis. Autologous PRP injection was safe and effective in this animal model; clinical trials are warranted to further investigate the use of PRP for ED management.

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