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Four Cardiac Hormones Increase Circulating Concentrations of Luteinizing Hormone and Testosterone

David L. Vesely, Gloria I. San Miguel, Imran Hassan, William R. Gower Jr., and Douglas D. Schocken

University of South Florida Cardiac Hormone Center, Departments of Biochemistry, Molecular Biology, Internal Medicine, Physiology and Biophysics, University of South Florida Health Sciences Center and James A. Haley Veterans Medical Center, Tampa, FL

This study was designed to determine whether four peptide hormones consisting of amino acids 1–30—long-acting natriuretic hormone (LANH), 31–67 (vessel dilator), 79–98 (kaliuretic hormone), and 99–126 (atrial natriuretic hormone [ANH])—of the 126 amino acid atrial natriuretic prohormone increase the circulating concentration of testosterone in healthy humans ($n = 30$). Vessel dilator, kaliuretic hormone, LANH, and ANH increased the circulating concentration of testosterone 3.8, 2.6, 3.9, and 3.4-fold, respectively ($p < 0.01$ for each), when infused at 100 ng/(kg of body wt·min) for 60 min. The increases in testosterone lasted 2.5–3 h after cessation of the respective atrial natriuretic peptides' infusions. ANH, vessel dilator, LANH, and kaliuretic hormone increased luteinizing hormone (LH) 3- to 8.4-fold ($p < 0.001$) during infusion, with the maximal increase in LH being 6.7- to 11.7-fold ($p < 0.001$) secondary to these cardiac hormones. Vessel dilator and kaliuretic hormone increased LH before increasing testosterone in a sequential fashion. These data suggest that four peptide hormones—ANH, LANH, vessel dilator, and kaliuretic hormone—increase the circulating con-centrations of LH and testosterone in humans.

Key Words: Atrial natriuretic peptides; testosterone; luteinizing hormone.

Introduction

Testosterone stimulates the synthesis of the atrial natriuretic hormone (ANH) prohormone in cultured rat atrial myocytes *(1)*. Testosterone also increases ANH prohormone gene expression when given to rats in vivo for 7 d *(2)*. The products of this prohormone's gene expression are four peptide hormones consisting of amino acids 1–30 (proANH 1–30; long-acting natriuretic hormone [LANH]), 31–67 (proANH 31–67; vessel dilator), 79–98 (proANH 79–98; kaliuretic hormone), and 99–126 (proANF 99–126; ANH) of the 126 amino acid prohormone *(3)*.

On the other hand, one of the products of this gene expression, ANH, stimulates testosterone production in vitro in mouse *(4–7)* and rat *(8)* Leydig cells. There has been one investigation of the effects of ANH on testosterone concentration in humans *(9)*. In this study, ANH significantly ($p < 0.01$) increased spermatic vein testosterone levels *(9)*. ANH is part of a peptide hormonal system that contains several peptide hormones from the same 126 amino acid prohormone with similar blood pressure (BP)-lowering, natriuretic, diuretic, and/or kaliuretic (i.e., potassium-excreting) properties *(10–18)*. LANH, vessel dilator, and kaliuretic hormone, which each circulate *(19,20)* and are released simultaneously with ANH *(21)*, have never been investigated with respect to their ability to modify the circulating concentration of testosterone.

These four cardiac peptide hormones are released from the heart with increased blood volume *(21)* and with strenuous exercise *(22)*. They increase in circulation in any pathophysiologic condition associated with salt and water retention *(19,23–27)*. Thus, these hormones increase in the circulation in congestive heart failure *(19),* in cirrhosis with ascites *(23,24),* and with acute renal failure *(25–27)* in an apparent attempt to overcome the salt and water retention present in these diseases.

The present investigation was designed to determine whether infusion of ANH, vessel dilator, LANH, and/or kaliuretic hormone modifies the circulating concentration of testosterone in humans. When each of these peptide hormones was found to increase the circulating concentration of testosterone, the concentration of luteinizing hormone (LH) was measured in the same plasma samples to help determine whether the increase in the circulating concentration of testosterone was owing to a direct effect (or effects) of the atrial natriuretic peptides (ANPs) on the testes and/or possibly secondary to an increase in LH. LH is the main known stimulus for testosterone production *(28)*.

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Author to whom all correspondence and reprint requests should be addressed: Dr. David L. Vesely, Director, Atrial Natriuretic Peptides Research Laboratories, James A. Haley Veterans Hospital-151, 13000 Bruce B. Downs Boulevard, Tampa, FL 33612. E-mail: vesely.david_l@tampa.va.gov

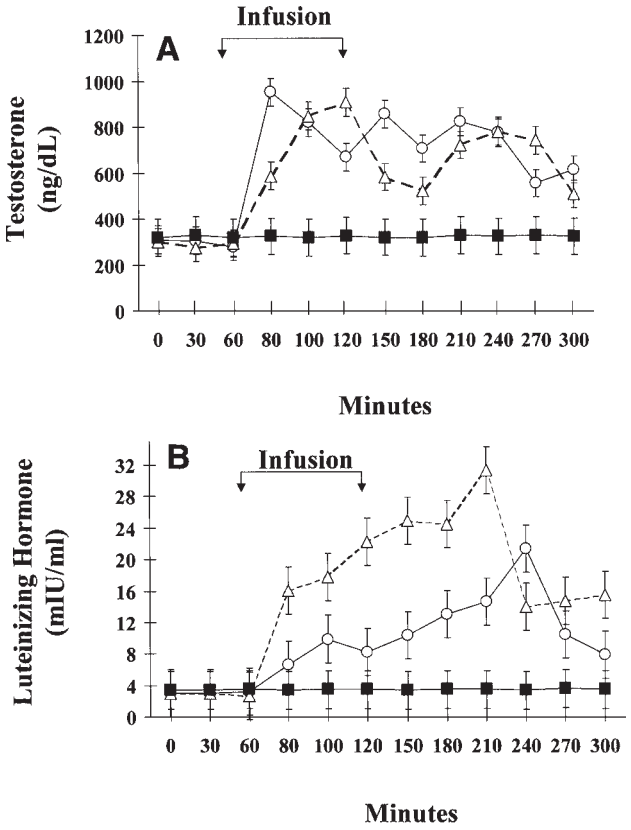

Fig. 1. (**A**) ANH (○) and kaliuretic hormone (△) increase the circulating concentration of testosterone when infused at 100 ng/(kg of body wt·min) for 60 min. The circulating concentrations of testosterone were significantly ($p < 0.05$) increased within 20 min of beginning the ANH infusion and at 40 min of beginning the kaliuretic hormone infusion compared with their preinfusion values when evaluated by analysis of variance (ANOVA). There was no significant increase in testosterone with the infusion of vehicle (■; 0.9% saline) when evaluated by ANOVA. (**B**) ANH (○) and kaliuretic hormone (△) increase the circulating concentration of LH when infused at 100 ng/(kg·body wt) for 60 min. ANH increased plasma LH concentrations significantly ($p < 0.05$) within 20 min of starting infusion and was maximal ($p < 0.001$) 2 h after cessation compared with its preinfusion values when evaluated by ANOVA. Kaliuretic hormone increased LH ($p < 0.001$) within 20 min of starting infusion compared with the LH preinfusion values and with vehicle alone (■; 0.9% saline) when evaluated by ANOVA. There was no significant change in LH with vehicle alone. $n = 6$ for each group.

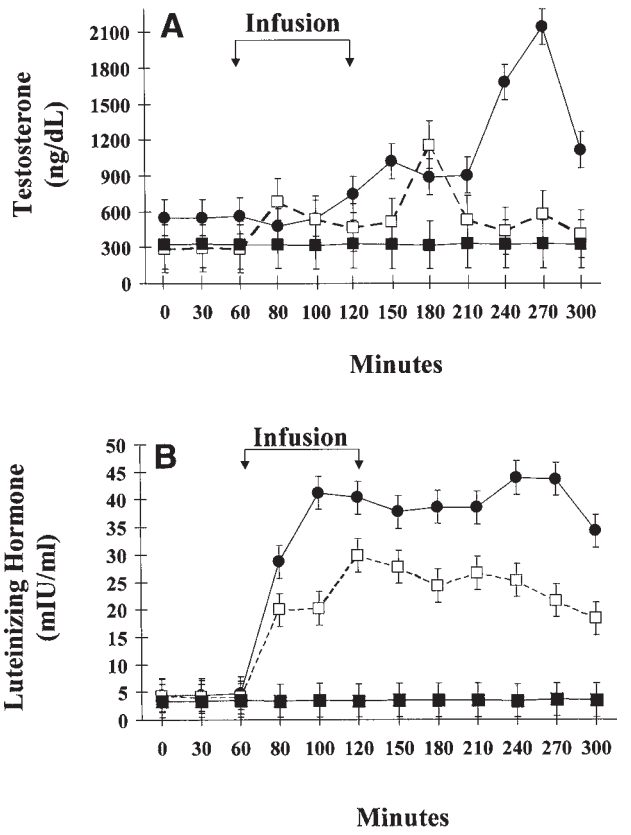

Fig. 2. (**A**) Vessel dilator (●) and LANH (□) increase the circulating concentration of testosterone when infused at 100 ng/(kg·body wt) for 60 min. The increase in testosterone secondary to vessel dilator was significant ($p < 0.05$) within 30 min of stopping the infusion of vessel dilator. The increase in testosterone secondary to LANH was significant ($p < 0.05$) within 20 min of beginning infusion when evaluated by ANOVA. There was no significant increase in testosterone with vehicle (■; 0.9% saline) alone. (**B**) Vessel dilator (●) and LANH (□) increase the circulating concentration of LH when infused at 100 ng/(kg·body wt) for 60 min. LH was significantly increased ($p < 0.001$) at the end of the vessel dilator and LANH infusions compared with vehicle (■; 0.9% saline) when evaluated by ANOVA. There was no significant change in LH secondary to vehicle alone. $n = 6$ for each group.

Results

ANH increased the circulating concentration of testosterone 3.4-fold ($p < 0.01$) during the first 20 min of infusion (Fig. 1A). ANH's effects on circulating testosterone concentrations were sustained, with testosterone 2.2-fold ($p \leq 0.05$) increased 3 h after the ANH infusion was stopped (Fig. 1A). Kaliuretic hormone increased the circulating concentration of testosterone 2.4-fold ($p < 0.05$) at the end of infusion and was 2.2-fold ($p \leq 0.05$) increased above its baseline concentration 2 h postinfusion (Fig. 1A). There was no increase in testosterone in the control group, which received vehicle (0.9% saline) alone (Figs. 1A and 2A).

Vessel dilator increased testosterone 1.8-fold ($p \leq 0.05$) 30 min after stopping infusion (Fig. 2A). Testosterone remained 3.8-fold ($p < 0.01$) increased in the circulation 2.5 h after the vessel dilator infusion ceased. LANH increased testosterone 2.3-fold ($p \leq 0.05$) within the first 20 min of infusion (Fig. 2A). The maximal increase in testosterone of 3.9-fold ($p < 0.01$) in the circulation occurred 1 h after the LANH infusion ceased (Fig. 2A). The order of maximal increases in testosterone secondary to the ANPs was as follows: LANH > vessel dilator > ANH > kaliuretic peptide. Testosterone was also measured in the urine of each of the subjects to help discern whether a decreased excretion of testosterone in the urine secondary to the respective ANPs might be the cause of the increased concentration of testos-

terone in the circulation after their infusion. There was no change in excretion of testosterone into the urine secondary to these ANPs (data not shown). These peptide hormones do not increase glomerular filtration rate at their respective concentrations utilized in our investigation *(13)*.

ANH increased plasma LH concentration twofold ($p \leq 0.05$) within 20 min of starting infusion (Fig. 1B). The increase in LH was maximal (6.7-fold increase; $p < 0.001$) 2 h after cessation of ANH infusion (Fig. 1B). Kaliuretic hormone increased the circulating concentration of LH 6-fold ($p < 0.001$) within 20 min of starting infusion and LH was increased 8.3-fold ($p < 0.001$) at the end of kaliuretic hormone infusion (Fig. 1B). Three hours after cessation of the kaliuretic hormone infusion, LH was increased 5.7-fold (Fig. 1B). The infusion of vehicle (0.9% saline) for 60 min did not result in any increase or decrease in LH (Figs. 1B and 2B).

Vessel dilator increased LH 6.1-fold ($p < 0.001$) within 20 min of starting infusion (Fig. 2B). The maximal increase in LH (9.2-fold; $p < 0.001$) occurred at 2 h postinfusion, and LH was increased 7.1-fold ($p < 0.001$) 3 h after cessation of the vessel dilator infusion. LANH increased the circulating concentration of LH fivefold ($p < 0.001$) within 20 min of beginning infusion (Fig. 2B). LH was increased 6.6-fold ($p < 0.001$) and 4.5-fold ($p < 0.01$) 2 and 3 h after stopping the LANH infusion, respectively (Fig. 2B). During the infusion of these peptide hormones, there was not any change in heart rate *(14)*. Both systolic and diastolic BP significantly decreased ($p < 0.05$) during the infusion of each of the respective peptide hormones *(14)*.

Discussion

ANH, LANH, vessel dilator, and kaliuretic hormone each increased the circulating concentration of testosterone in human volunteers. In the one previous investigation of ANPs in humans, ANH increased the spermatic vein concentrations of testosterone without modifying the secretion of gonadotropin *(9)*. The other three cardiac peptide hormones synthesized within the same gene as ANH were not investigated in this previous study *(9)*, and they have not been investigated previously in any study with respect to the metabolism of testosterone.

Each of the four infused peptide hormones modified the circulating concentrations of testosterone, but their onset of action and ability to maximally enhance testosterone were different. ANH and LANH had a more rapid onset of action, with testosterone significantly increased ($p < 0.05$) at the first measured time point during their infusions (i.e., at 20 min). Vessel dilator and kaliuretic hormone, on the other hand, were slower in onset of action with neither increasing testosterone in the first 20 min of their infusions. Vessel dilator's maximal-induced increases in circulating testosterone levels (3- to 3.8-fold) were at 2 and 2.5 h postinfusion, when the effects of the three other ANPs on testosterone concentrations had begun to wane. These data suggest an intricate relationship among these four cardiac hormones, which are released simultaneously with physiologic stimuli *(21),* in modulating testosterone production. ANH and LANH increased testosterone in the circulation within 20 min, and vessel dilator sustained these effects significantly for 2–2.5 h (Figs. 1 and 2).

Whether vessel dilator, LANH, kaliuretic hormone, and/or ANH were directly stimulating the testes to secrete testosterone into the circulation could not be determined definitively in our in vivo study. Previous in vitro studies have shown that ANH can directly stimulate the release of testosterone *(4–8)* mediated through the intracellular messenger cyclic guanosine 5'-monophosphate (cGMP) *(6,7,29,30)*. Schumacher et al. *(29)* have further defined the mechanism of how ANH-induced testosterone secretion is mediated via demonstrating that cGMP activates cyclic adenosine monophosphate (cAMP)-dependent protein kinase and displaces cAMP from its intracellular binding sites in mouse Leydig cells. Vessel dilator, LANH, and kaliuretic hormone, likewise, increase the intracellular mediator cGMP *(10,31)*. In purified mouse *(30)* and rat *(32)* Leydig cells, ANH stimulates the production of intermediate precursors of testosterone biosynthesis including progesterone, 17α-hydroxyprogesterone, androstenedione, pregnenolone, 17α-hydroxypregnenolone, and dehydroepiandosterone sulfate, beginning at the cholesterol side-chain cleavage enzyme. Leydig cells contain receptors for ANH *(30)*. Thus, it is possible that these peptide hormones have direct effects on the testes via stimulation of testosterone synthesis and/or release from Leydig cells. The fact that BP decreased during the infusion of these peptide hormones suggests that increased perfusion of testes and pituitary were not the cause of the enhanced secretion of testosterone and LH, respectively.

Our data suggest that the ability of these peptide hormones to induce an increase in testosterone may also be secondary to their ability to increase LH. There was an initial increase in LH followed sequentially by an increase in testosterone in response to vessel dilator and kaliuretic hormone. These observations suggest that the increase in testosterone observed secondary to these hormones may be owing to their ability to stimulate the release of LH, which may have caused the increase in testosterone noted within the circulation. The ability of these cardiac hormones to increase LH correlates directly with the in vitro study of Horvath et al. *(33)*, in which 0.01 and 1 μ*M* ANH caused a dose-related increase in the release of LH from isolated rat pituitary cells. Samson et al. *(34)* have found that infusion of ANH for 30 min may have a paradoxic effect of decreasing LH in the circulation of orichidectomized rats but not in male rats that have not been orichidectomized. There is controversy regarding the rat pituitary studies, since Abou-Samra et al. *(35)* have suggested that the Bachem® preparation of ANH used by Horvath et al. *(33)* may have been contaminated with a LH-releasing agonist since they

Table 1
Mean Baseline Age, Weight, BP, and Heart Rate of Control Subjects and Subjects Receiving Vessel Dilator, LANH, Kaliuretic Hormone, and ANH[a]

	Age (yr)	Weight (kg)	BP (mmHg)	Heart rate (beats/min)
Control subjects	33 ± 4	76 ± 6	110 ± 4/71 ± 4	68 ± 2
LANH	31 ± 4	76 ± 6	103 ± 3/66 ± 2	68 ± 3
Vessel dilator	35 ± 5	75 ± 5	114 ± 5/72 ± 3	64 ± 2
Kaliuretic hormone	32 ± 5	76 ± 6	108 ± 5/72 ± 5	66 ± 3
ANH	33 ± 6	76 ± 4	109 ± 4/75 ± 2	70 ± 4

[a] The control subjects received 20 mL 0.9% saline infused over 1 h as a control for the sodium content and volume of the peptide infusion. The respective peptides were dissolved in 20 mL of 0.9% saline, which was infused over 60 min. There was no significant difference in the age, weight, heart rate, or BP of the individuals in the five groups when evaluated by repeated measures of ANOVA. $n = 6$ for each group. This table is a summary of subjects utilized in ref. *13*.

(35) found that some of the Bachem ANH preparations were contaminated. It is, therefore, important to underscore that ANH from Peninsula (Belmont, CA) utilized in the present investigation was found by the same authors not to be contaminated with an LH–releasing hormone agonist *(34,35)*.

Materials and Methods

Experimental Subjects

Thirty healthy men ages 20–58 (average: 32 yr) all normotensive with BPs <125/80 mmHg were studied. The subjects had heart rates ranging from 56 to 80 beats/min, with respiration rates between 12 and 14/min. They were divided into five similar groups with six individuals in each group. Table 1 provides the age, weight, BP, and heart rate for each group. None of the volunteers had any known disease and none were taking any medication. Written informed consent was obtained from each of the volunteers after the studies were fully explained. This study was approved by the Institutional Review Board of the University of South Florida Health Sciences Center and the Research Committee of the James A. Haley Veterans Hospital and followed the guidelines of the Declaration of Helsinki. This study was also approved by the US Food and Drug Administration (FDA IND 32,119). All the healthy study subjects had participated previously in a study of the natriuretic, diuretic, and BP-lowering effects of LANH, vessel dilator, kaliuretic hormone, and ANH *(13)*.

Experimental Protocol

After obtaining written informed consent, a 20-gage, 1.5-in. Insyte-w catheter was placed into the forearm of each subject for infusion and blood sampling. A 60-min baseline period preceded any infusion. A total volume of 20 mL of normal saline (0.9% NaCl, with or without peptides) was infused by a constant-rate infusion pump over a 60-min period. Blood samples were obtained every 20 min during the infusion and at 30-min intervals during the 1-h baseline and 3-h postinfusion periods. Thus, one group (control group or sham infusion) received only 20 mL of normal saline without any of the peptide hormones, and the other four groups received one of the respective peptide hormones in 20 mL of normal saline. The infusion of 100 ng/(kg of body wt·min) of these ANPs was chosen because the rate of release of the N-terminal ANH prohormone peptides from the atrium of the heart with physiologic stimuli is 138–292 ng/(kg of body wt·min), whereas the release rate of ANH from the atrium is 76 ng/(kg of body wt·min) *(36)*. All subjects were studied in the morning after an overnight fast, beginning their baseline period at 8:00 AM. Each volunteer was studied in the seated position and received only one peptide hormone infusion. Molar equivalents of the 100 ng/kg of body wt dose are 32, 29, 26, and 46 pmol/(L·kg of body wt) for ANH, LANH, vessel dilator, and kaliuretic hormone, respectively. The 60-min time period in Figs. 1 and 2 (which is the time period immediately before one of the respective infusions was begun) serves as the control (baseline) value with which one can compare any effects observed at later time points in this investigation. The change in testosterone and/or LH is the amount of increase in testosterone or LH compared with the respective preinfusion measurement (i.e., 60-min time period) in each subject. The control group consisting of individuals who received 20 mL of 0.9% saline (vehicle) was added to demonstrate that testosterone and LH do not change by chance alone during the time period used in this investigation.

Purity of Atrial Natriuretic Hormones

The human forms of LANH, vessel dilator, kaliuretic hormone, and ANH were synthesized by Peninsula. Before their use in these studies, samples of these commercially synthesized peptides were subjected to high-performance liquid chromatography to determine their purity using a Novapak C_{18} (5-μm) cartridge column (Waters Chromatography Division, Millipore, Milford, MA).

Measurement of Testosterone

Testosterone was measured utilizing a solid-phase ^{125}I radioimmunoassay (RIA) from Diagnostic (Los Angeles, CA). In this assay, testosterone is measured directly without extraction or predilution. Testosterone was measured in serum samples at 0, 30, 60 (beginning of respective peptide hormone infusions), 80, 100, 120 (end of peptide infusions), 150, 180, 210, 240, 270, and 300 min in each subject ($n = 30$). In this solid-phase RIA, polypropylene tubes with antibodies to testosterone (Coat-A-Count®) are utilized in which ^{125}I-labeled testosterone competes for 3 h at 37ºC within the respective healthy volunteers' samples for antibody sites. The detection limit is approx 4 ng/dL (0.14

nmol/L). The lower limit of normal testosterone for healthy adult men age 50 yr and older is 181 ng/dL, while the lower limit of healthy adult men ages 20–49 yr is 262 ng/dL. This assay is highly specific for testosterone with the following crossreactivities: aldosterone, 0%; 5β-androstan-3α, 17β-diol, 0.4%; androstenedione, 0.5%; 5α-androstan-3β, 17β-diol, 0.04%; 5-androsten-3β, 17β-diol, 0.2%; 5α-androstan-3, 17-dione, 0.05%; androsterone, 0.004%; corticosterone, 0.002%; cortisol, 0.005%; cortisone, 0.02%; danazol, 0.09%; 11-deoxycortisol, 0%; dexamethasone, 0.003%; DHEA, 0.002%; DHEA-sulfate, 0.006%; 5α-dihydrotestosterone, 3.3%; estradiol, 0.02%; estrone, 0.01%; ethisterone, 0.7%; fluoxymesterone ("halotestin"), 0.01%; 19-hydroxyandrostenedione, 2.0%; methyltestosterone, 1.7%; norethindrone, 0.1%; prednisone, 0%; progesterone, 0%; progesterone, 0%; spironolactone, 0%; 11β-hydroxytestosterone, 0.8%; and triamcinolone, 0.2%. The intra- and interassay coefficients of variation (CVs) for the testosterone assay were 5.0 and 7.9%, respectively. Sex-binding globulin has no significant effect on the Coat-A-Count total testosterone assay. Serial dilution has revealed excellent parallelism (93–103%) of standards and unknowns in this assay. The samples for the testosterone and LH measurements were aliquots that had been separated from the sample collected at the time of investigation. These aliquots were stored in a –80ºC freezer and thawed only at the time of measurement of LH and testosterone.

Measurement of LH

LH was measured with a solid-phase immunoradiometric assay (IRMA) from Diagnostic in the samples collected from 0 to 300 min (n = 30). This LH IRMA assay is performed similarly to the testosterone assay except that the samples are incubated for 1 h instead of 3 h for the testosterone assay. It can detect as little as 0.15 mIU/mL of LH. The LH IRMA assay's antibodies are highly specific for LH with crossreactivities of structurally related glycoproteins of 0.0002% for follicle-stimulating hormone, 0.003% for thyroid-stimulating hormone, and 0.0005% for human chorionic gonadotropin. There is excellent parallelism (93-108%) of standards and unknowns in this assay. The intra- and interassay CVs were 1.3 and 3.4%, respectively. The normal range of LH in this assay for healthy adult men is 0.4–5.7 mIU/mL.

Statistical Analyses

The data obtained are illustrated as the mean ± SE. Differences in testosterone and LH measurements between subjects or groups of subjects were evaluated by the one-way ANOVA. Measurements of testosterone and LH obtained in the same subjects over time were evaluated by repeated measures of ANOVA. To be considered statistically significant, we required a probability value up to <0.05 (95% confidence).

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