Puberty alters renal water handling

B. Mahler, K. Kamperis, C. Ankarberg-Lindgren, J. Frokijer, J. C. Djurhuus, and S. Rittig

1Department of Pediatrics, Aarhus University Hospital, Aarhus, Denmark; 2Pedictric Growth Research Center, Gothenburg, Sweden; 3The Water and Salt Research Center, Aarhus University Hospital, Aarhus, Denmark; and 4The Institute of Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark

Submitted 24 May 2013; accepted in final form 24 October 2013

Mahler B, Kamperis K, Ankarberg-Lindgren C, Frokijer J, Djurhuus JC, Rittig S. Puberty alters renal water handling. Am J Physiol Renal Physiol 305: F1728–F1735, 2013. First published October 30, 2013; doi:10.1152/ajprenal.00283.2013.—We investigated the influence of sex and puberty stage on circadian urine production and levels of antidiuretic hormone [arginine vasopressin (AVP)] in healthy children. Thirty-nine volunteers (9 prepuberty boys, 10 prepuberty girls, 10 midpuberty boys, and 10 midpuberty girls) were included. All participants underwent a 24-h circadian inpatient study under standardized conditions regarding Na+ and fluid intake. Blood samples were drawn every 4 h for measurements of plasma AVP, serum 17β-estradiol, and testosterone, and urine was fractionally collected for measurements of electrolytes, aquaporin (AQP)2, and PGE2. We found a marked nighttime decrease in solute-free water reabsorption (TcH2O) (39). This renal effect of AVP is mediated through AVP-mediated activation of V2 receptors of collecting ducts principal cells, which leads to the insertion of aquaporin (AQP)2 channels in the apical membrane and thereby increases solute-free water reabsorption (TcH2O). However, despite similar levels of plasma AVP, differences between boys and girls in urine output regulation have not been investigated in healthy children. Thus, studying children before and after the onset of puberty offers a unique chance to investigate the impact of sex and sex hormones on renal water handling. This seems of particular importance in clinical settings such as enuresis nocturna, where lower circadian plasma AVP levels have been demonstrated in children (27) and a high rate of spontaneous resolution of bedwetting occurs during childhood (16). The regulation of renal water excretion is closely linked to AVP-mediated activation of V2 receptors of collecting ducts principal cells, which leads to the insertion of aquaporin (AQP)2 channels in the apical membrane and thereby increases solute-free water reabsorption (TcH2O). The renal effect of AVP is antagonized by PGE2 at several different levels (10, 13), making the urinary excretion of AQP2 and PGE2 interesting markers when evaluating the urinary concentration effect of AVP.

The aim of this study was to investigate the influence of sex and puberty stage on circadian rhythm in diuresis, AVP secretion, and renal water handling as well as urinary AQP2 and PGE2 excretion in healthy children during a standardized fluid and Na+ intake regimen. The girls included were all premenarche to avoid previously described AVP changes during the menstrual cycle (11).

MATERIALS AND METHODS

The study protocol was approved by the regional Committee on Biomedical Research Ethics, and informed consent was obtained from all participants and their parents. This study was performed according to “Guidelines for Good Clinical Practise.”

Address for reprint requests and other correspondence: B. Mahler, Dept. of Pediatrics, Regionshospitalle Randers, Skovlyvej 1, Randers 8930, Denmark (e-mail: mahler@ki.au.dk).
Age, yr 8.2
Body weight, kg 28.1 ± 4.7
Height, cm 131.4 ± 6.6
Body surface area, m² 1.01 ± 0.11
Maximum voided volume, ml 286.7 ± 98.2
Breast stage* 1 2 (1–2)
Testicle size, ml* 2 (1–2) 12 (8–15)
Number of children/group 9 9 10 10

Values are means ± SD except for the characteristics indicated (*), where median and range (in parentheses) are given. Bladder capacity was estimated using the maximum voided volume and was found to be within the normal range for age.

Study subjects. Thirty-nine healthy volunteers were recruited through hospital staff members. Inclusion criteria for children in prepuberty were an age of 7–8 yr, Tanner stage 1 (girls: breast stage 1; boys: testicle volume 1–2 ml), and for children in midpuberty an age of 12–15 yr, Tanner stage 3–4 (girls: breast stages 2–3, premenarche; boys: testicle volume 8–15 ml). Children had a normal physical examination, including blood pressure measurements. Height and weight were within 2 SDs of normal growth (40). Children had complete bladder emptying upon voiding (determined by postvoid ultrasound) and normal urine dipstick analysis. There was no history of day or night urinary or fecal incontinence after the age of 4 yr and no known history of prior severe illnesses or use of any medication, drugs, alcohol, or tobacco.

Study design. The experimental procedure was a 24-h inpatient study under standardized conditions for comparisons of diuresis and hormone profiles between sex and puberty stage groups.

Participants were admitted to the Department of Pediatrics the night before the study for accommodation to the environment. On the morning of the study, an intravenous, heparinized catheter was inserted into a cubital vein for blood sampling. During the experimental period, diet and fluid intake were standardized as directed by a clinical dietician (Na⁺: 3 mmol/kg body wt and water: 25 ml/kg body wt divided in 2/3 before 16.00 hours and 1/3 until bedtime). Meals were served at 08.00, 12.00, and 18.00 hours, and caffeine-containing beverages and additional servings were not allowed. Activity was allowed between 08.00–22.00 hours, and bedtime was set to no later than 22.00 hours and noted for each child. Sleep during daytime was not allowed. Children were supervised by an adult during the entire period. Blood samples were drawn every 4 h starting at 08.00 hours. Participants were in bed and in the supine position at least 15 min before each blood sampling, and during the night care was taken not to disturb the children’s sleep. To prevent clotting, the intravenous catheter was flushed with 10 ml isotonic saline and 0.25 ml heparin before each blood sampling, and during the night care was taken not to disturb the children’s sleep. To prevent clotting, the intravenous catheter was flushed with 10 ml isotonic saline and 0.25 ml heparin (50 IE). Blood samples were transported on ice, centrifuged at +4°C, and stored at −20°C unless immediately analyzed. The amount of blood drawn was 2 ml at 08.00 hours and 19 ml at subsequent blood samples. Analyses of creatinine and osmolality were performed for each blood sample. Blood samples from 12.00 hours, and subsequent sampling time points were analyzed for plasma concentrations of AVP.

Urine was fractionally collected in 4-h intervals during daytime, starting after bladder emptying at 08.00 hours. Nighttime urine collection started at 22.00 hours until the end of the study at 08.00 hours. All voidings were spontaneous except after each blood sampling and before bedtime where the participants were asked to empty their bladder. The urine volume was measured, and the concentration of creatinine as well as osmolality was determined. Aliquots were stored at −80°C for analyses of PGE₂ and AQP2. Leupeptin (60 μl, 0.05 mg/ml) and NaN₃ (100 μl, 100 mM) were added to each 3-ml aliquot of urine for AQP2 analyses before storage, a modification of the urine storage method recommended by the National Institutes of Health Uroprotein Committee (36).

Home recordings of 48-h fluid intake and micturition pattern (time and volume) as well as the collection of diuresis in 2-night and 2-day portions were made during a weekend after the admission. Bedtime and time of awakening were also noted. Urine was refrigerated and delivered to the laboratory within 24 h after the end of the collection period. There were no restrictions regarding fluid or food intake, activity level, or hours of sleep.

Biochemical determinations. Plasma and urine osmolalities were determined using the freezing point depression method (Osmometer 3900, Advanced Instruments). Creatinine measurements were carried out at the Department of Clinical Biochemistry; plasma and urine analyses were performed on a Vitros 950 analyzer.

AVP was measured in plasma after extraction in a Sep-Pak Plus C18 cartridge (Waters, Milford, MA) using a previously described radioimmunoassay (RIA) (15). The highly specific AVP antibody
Circadian rhythm was tested using Student’s t-test for the effect of sex and puberty stage. Furthermore, plasma AVP and plasma osmolality were tested in the same model for the effect of serum estradiol and serum testosterone. Pearson’s test was used for correlation analysis. All analyses were performed using STATATA 10.0 software (STATATA). Results are given as means ± SE. Statistical significance was defined at \( P < 0.05 \).

RESULTS

Demographics. One prepuberty girl was excluded due to the occurrence of an enuretic episode during the inpatient study. Baseline characteristics of participants are shown in Table 1. Sex hormone levels are shown in Fig. 1.

Inpatient circadian experiments. Overall, all groups of children had a pronounced circadian rhythm in diuresis (Table 2) with decrease in nighttime urine output and reciprocal changes in urine osmolality (Fig. 2). Day-to-night ratios of urine output and osmolality were not different between sex or puberty stage.

Table 2. Urine output parameters presented as the ratio between day and night values in groups defined by sex and puberty stage

<table>
<thead>
<tr>
<th></th>
<th>Day-to-Night Ratio</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
</tr>
<tr>
<td>Admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start of data collection</td>
<td>1.89 ± 0.30</td>
<td>2.47 ± 0.38</td>
<td>2.25 ± 0.34</td>
</tr>
<tr>
<td>Urine flow rate</td>
<td>0.68 ± 0.11</td>
<td>0.55 ± 0.07</td>
<td>0.71 ± 0.13</td>
</tr>
<tr>
<td>Glomerular filtration rate</td>
<td>0.93 ± 0.11</td>
<td>0.90 ± 0.08</td>
<td>1.00 ± 0.04</td>
</tr>
<tr>
<td>Solute-free water reabsorption axis</td>
<td>0.79 ± 0.16</td>
<td>0.57 ± 0.11</td>
<td>0.70 ± 0.16</td>
</tr>
<tr>
<td>Clearance of osmoles</td>
<td>1.14 ± 0.14</td>
<td>1.23 ± 0.13</td>
<td>1.26 ± 0.10</td>
</tr>
<tr>
<td>Urine AQP2</td>
<td>1.36 ± 0.12</td>
<td>1.60 ± 0.33</td>
<td>1.67 ± 0.21</td>
</tr>
<tr>
<td>Urine PGE2</td>
<td>1.57 ± 0.18</td>
<td>1.64 ± 0.14</td>
<td>1.31 ± 0.16</td>
</tr>
<tr>
<td>Number of children/group</td>
<td>9</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Home</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start of data collection</td>
<td>1.60 ± 0.11</td>
<td>2.07 ± 0.25</td>
<td>1.85 ± 0.23</td>
</tr>
<tr>
<td>Urine flow rate</td>
<td>0.88 ± 0.07</td>
<td>0.69 ± 0.06</td>
<td>0.86 ± 0.10</td>
</tr>
<tr>
<td>Urine AQP2</td>
<td>2.23 ± 0.34</td>
<td>2.46 ± 0.55</td>
<td>3.77 ± 1.36</td>
</tr>
<tr>
<td>Urine PGE2</td>
<td>2.03 ± 0.19</td>
<td>1.94 ± 0.23</td>
<td>1.61 ± 0.17</td>
</tr>
<tr>
<td>Number of children/group</td>
<td>9</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Circadian variation was detected for all parameters, and no significant changes between groups were detected except a significantly lower day-to-night ratio of urine PGE2 in midpuberty boys on home registrations. AQP2, aquaporin 2. *\( P < 0.05 \); †\( P < 0.001 \); ‡not significant.
We did not find any circadian rhythm in estimated GFR, and there were no differences in day-to-night ratios of estimated GFR between sex and puberty groups (Table 2).

AQP2 excretion showed pronounced circadian rhythm with decreased nighttime urinary excretion. This was observed in all groups with no effect of sex and puberty stage (Table 2). Nighttime AQP2 excretion differed significantly between groups ($P = 0.05$), with higher AQP2 excretion in prepuberty ($278 \pm 23 \text{ pg·kg}^{-1} \text{·h}^{-1}$) compared with midpuberty ($194 \pm 17 \text{ pg·kg}^{-1} \text{·h}^{-1}$, $P < 0.01$). There were no sex differences ($P = 0.82$; Fig. 2).

PGE2 excretion decreased during nighttime, with a significant circadian rhythm in urinary PGE2 excretion in all groups (Table 2). We did not observe differences in the day-to-night ratio of PGE2 excretion between groups, and there were no significant differences in the levels of nighttime urinary PGE2 excretion ($P = 0.120$; Fig. 2).

Plasma AVP showed a pronounced circadian rhythm with peak plasma AVP levels at midnight in all groups ($P < 0.001$; Fig. 3). Increasing levels of plasma AVP were significantly associated with an increase in $T_c H_2O$ [$\alpha = 0.94 (0.45–1.43)$, $P < 0.001$], but no association between the excretion of AQP2 and plasma AVP was found ($P = 0.106$). There was, as expected, a strong correlation between urine osmolality and urine AQP2 concentration, but this correlation was independent of sex ($P = 0.487$) and puberty stage ($P = 0.313$; Fig. 4). Plasma osmolality showed a significant decrease from 08.00 to 12.00 hours ($P < 0.001$), but, overall, there were no differences between day and night levels ($P = 0.173$). Girls had significantly higher plasma osmolarity [0.7% (0.1–1.3)] compared with boys ($P = 0.05$; Fig. 3). Estradiol and testosterone levels were not associated with the level of plasma osmolality (estradiol: $P = 0.70$, testosterone: $P = 0.31$).

Home recordings. All children demonstrated pronounced circadian rhythms in urine flow and urine osmolality during home registrations (Table 2). When we compared inpatient and home recordings (Table 3), the total 24-h urine production was significantly lower in all four groups during home recording, but no differences between groups were observed. Urine osmolality measurements were comparable to values during admission, and no differences between groups were observed. Both PGE2 and AQP2 excretion showed pronounced circadian rhythms during home recordings with considerably lower nighttime levels. Day-to-night ratios of both parameters were, however, significantly higher than during the inpatient study (Table 2). When we analyzed AQP2 excretion in urine from...
was similar during the 2 home registration nights ($P = 0.83$) but significantly lower than during the inpatient study night ($P < 0.01$). Moreover, we found significant differences between groups in nighttime PGE2 excretion ($P_{\text{group}} < 0.01$) with no effect of puberty ($P_{\text{puberty}} = 0.27$) but significantly lower PGE2 excretion in girls compared with boys (excretion of PGE2: $130 \pm 44 \text{ pg·kg body wt}^{-1} \cdot \text{h}^{-1}$ in girls and $168 \pm 50 \text{ pg·kg body wt}^{-1} \cdot \text{h}^{-1}$ in boys, $P < 0.05$).

**DISCUSSION**

This study is, to our knowledge, the first investigation of the effect of sex and puberty stage on circadian water balance regulation in a population of healthy children. We confirmed the circadian rhythm of urine production and found that the circadian variation in plasma AVP persisted into puberty and remained uninfluenced by sex. There was, however, a developmental change in the renal handling of free water as the nocturnal reduction in urine production seemed more dependent on free water reabsorption during prepuberty compared with midpuberty.

The investigated variables were very sensitive to study conditions, especially hydration status and sleep. We compared the inpatient registrations with home registrations of fluid intake and measurements of urine output. On evaluation, we found no systematic differences between groups in fluid intake or urine output at home, and during the first 4 h of the experimental day, there was a huge interindividual variation in urine osmolality but no systematic tendency or any significant differences between groups. Despite these reservations concerning investigational circumstances, we found that the inpatient setup can be used to describe the circadian rhythm in osmoregulation, and our standardized intake protocol is close to the conditions of everyday life for healthy children.

The AVP-V2R-AQP2 axis regulates the ability of the kidneys to reabsorb solute-free water. The main trigger for AVP secretion is plasma tonicity. In this study, girls shared higher plasma osmolality levels compared with boys regardless of puberty stage (Fig. 3). Studies (18, 32) in adults have failed to show sex differences in plasma osmolality between low-estriadiol women and men, but high-estriadiol women tended to have lower plasma osmolality. In the present study, the observed higher plasma tonicity in girls compared with boys did not seem to result in increased AVP secretion as plasma AVP levels were similar. This fact gives rise to the hypothesis that there are differences in the setting of osmoregulation between boys and girls. Hydration status affects plasma osmolality and might explain the observed sex differences. However, we

---

**Figs. 3 and 4.** Circadian variation in the plasma levels of arginine vasopressin (P-AVP) and osmolality (P-osmolality) among participants in the sex and puberty groups. There was no effect of sex ($P = 0.56$) or puberty stage ($P = 0.72$) on the circadian changes or levels of plasma AVP. In addition, there was no effect of estradiol ($P = 0.47$) or testosterone ($P = 0.98$) on levels of plasma AVP.

---

**Fig. 5.** Association between urine osmolality (in mosM/kg) and urine AQP2 (U-AQP2) concentrations (in pg/ml). Left: observations for boys and girls; right: observations in prepuberty and midpuberty groups.
found no indication of a systematic difference in hydration status during the study between boys and girls. Thus, 24-h urine production at home was without sex differences. We also found that all children had comparable urine osmolalities both in the first urine collection and average 24-h urine. The higher plasma tonicity in girls compared with boys was not affected by estradiol or testosterone levels. These results question the physiological importance of a single sex hormone effect on osmoregulation in these age groups and rather points toward a combined effect of sex with differences in receptor density (37) or brain regulation (19).

Based on the results of the present study, a circadian AVP rhythm is clearly present in healthy children aged 7–15 yr. Sex and puberty stage at this age do not seem to influence circadian AVP rhythm. It is still debatable whether a circadian AVP rhythm is less evident or even absent in adults, with studies providing conflicting data (17, 21, 26). If presumed that the circadian AVP rhythm is absent in adults, our data do not indicate that such changes occur during the first stages of puberty. As plasma osmolality was stable throughout the entire experimental period, other mechanisms must be considered as being responsible for the observed nighttime increase in plasma AVP secretion, with the suprachiasmatic nucleus (internal circadian clock), sleep, AVP-melatonin interactions, and the autonomous nervous system as possible candidates (34).

The higher levels of plasma AVP during nighttime in the 7- to 15-yr age group contribute significantly to the increased nocturnal renal water reabsorption. The renal tubules are sensitive to even minor changes in plasma AVP; changes in plasma AVP that are undetectable with the available assays seem to decrease diuresis and increase TH2O (4). The observed increase in nocturnal plasma AVP levels leads to considerably higher water reabsorption, and the association between TH2O and plasma AVP is in agreement with the theory of a physiological effect of the increasing nighttime level in plasma AVP. Sex or puberty stage did not influence the circadian rhythm of plasma AVP, day-to-night ratio of urine output, or TH2O; however, prepuberty children showed significantly higher nighttime TH2O than midpuberty children despite comparable levels of plasma AVP. This may indicate an increased sensitivity of the renal concentration mechanism in the first stages of puberty.

The excretion of AQP2 in animals has proved useful as a marker of AVP stimulation of AQP2 trafficking in distal tubules (39). Also, in a human intervention study (14), endogenously stimulated or suppressed AVP was correlated with an increase and a decrease in urine AQP2, respectively. We found a very strong correlation between urine osmolality and urinary AQP2 concentration in accordance with some noninterventional studies in healthy adults (8, 14), but sex or puberty stage did not affect this correlation. There was a circadian rhythm in AQP2 excretion with decreased nighttime excretion. Consistent with the changes in TH2O, we found a more pronounced AQP2 activation in the prepuberty group, with significantly higher levels of AQP2 excretion during nighttime despite comparable levels of AVP. The day-to-night difference with a decrease in AQP2 during nighttime was surprising, and the basic physiology behind AQP2 excretion and function needs to be further elucidated.

From this study, we conclude that the activation of the AVP-AQP2 axis and the increase in TH2O are of importance in the reduction of nighttime diuresis in healthy children. The nighttime effect of the activation of the AVP-AQP2 axis is more pronounced in prepuberty compared with midpuberty. Our finding of a developmental change in renal water handling from prepuberty to midpuberty once more raises the following question: is there a maturation of water homeostasis during childhood? Due to methodological considerations, we only investigated children from 7 to 15 yr old. A circadian rhythm in diuresis is present from the age of 3 yr in healthy children (22). It could be interesting to examine the development of the circadian rhythm in diuresis from birth throughout childhood.

The role of urinary PGE2 as a marker of renal water handling is debatable. PGE2 acts as negative feedback on AVP-stimulated water reabsorption (41). Animal studies (37, 38) have indicated that the antidiuretic effect of AVP is greater in males compared with females, an effect that can be abolished by cyclooxygenase inhibitors. We found, like previous investigators, a circadian rhythm in PGE2 excretion (1) with a decrease in nighttime levels. PGE2 excretion was not influenced by sex or puberty stage, and the nocturnal reduction was evident in all four study groups. Differences in PGE2 could not account for the observed differences in renal sensitivity to plasma AVP observed in the two stages of puberty.

The observed difference in TH2O between prepuberty and midpuberty children does not seem to be explained simply by testosterone or estradiol. It could be a combined effect of an increase in both sex hormones and the development of sex-specific characteristics in body composition. Intervention studies with either water load- or thirst-induced stimulation of the AVP-AQP2 axis have proven useful to detect sex differences in adults but could also be used in children for further exploration of the developmental changes in osmoregulation.

---

**Table 3. Urine output parameters from admission and home registration in all participants (total) and in subgroups defined by sex and puberty stage**

<table>
<thead>
<tr>
<th></th>
<th>Admission</th>
<th></th>
<th></th>
<th></th>
<th>Home</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepuberty</td>
<td>Midpuberty</td>
<td></td>
<td></td>
<td></td>
<td>Prepuberty</td>
<td>Midpuberty</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
<td>Girls</td>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine excretion rate, ml/kg body wt</td>
<td>34.7 ± 2.7</td>
<td>30.9 ± 1.8</td>
<td>29.7 ± 2.7</td>
<td>31.6 ± 3.12</td>
<td>31.7 ± 1.32†‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine osmolarity, mosM/kg</td>
<td>611 ± 45</td>
<td>537 ± 90</td>
<td>593 ± 67</td>
<td>488 ± 46</td>
<td>558 ± 30†‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children/group</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>38</td>
<td>10</td>
<td>10</td>
<td>38</td>
</tr>
<tr>
<td>Home</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine excretion rate, ml/kg body wt</td>
<td>29.1 ± 3.0</td>
<td>31.1 ± 3.9</td>
<td>24.2 ± 2.1</td>
<td>28.5 ± 3.15</td>
<td>28.0 ± 1.51*†‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine osmolarity, mosM/kg</td>
<td>730 ± 47</td>
<td>568 ± 78</td>
<td>696 ± 78</td>
<td>581 ± 75</td>
<td>654 ± 36*†‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children/group</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>6</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *Difference between admission and home registration (P < 0.05); †differences between groups; ‡not significant.
In conclusion, the main elements of renal water handling seem to be independent of sex but influenced by puberty stage, with higher nighttime $T_{H_2O}$ and an augmented AQP2 activation in prepuberty. Healthy children aged 7–15 yr share a circadian rhythm in diuresis and AVP secretion with nocturnal decrease in diuresis and reciprocal changes in the plasma levels of AVP. This rhythm is independent of sex and puberty stage.

GRANTS
This work was made possible by grants provided by the Fonden til Lægevidenskabens Fremme, Statens Forskningsråd for Sygdom og Sundhed, Karen Elise Jensen Foundation, and the University of Aarhus Research Foundation.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: B.T.M., K.K., C.A.-L., J.F., J.C.D., and S.R. conceived the study and designed the experiments; B.T.M. and K.K. performed the experiments; B.T.M. and K.K. analyzed the data; B.T.M., K.K., C.A.-L., J.F., J.C.D., and S.R. wrote the manuscript; B.T.M., K.K., C.A.-L., J.F., J.C.D., and S.R. edited and revised the manuscript; B.T.M., K.K., C.A.-L., J.F., J.C.D., and S.R. approved the final version of the manuscript.

REFERENCES


