ORIGINAL ARTICLE

Testosterone effects on pain and brain activation patterns

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Conflicts of interest
No competing interests declared.

Funding
This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2015R1D1A1A09059766), and was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (2016R1A2B3016609).

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Submitted 27 March 2017; accepted 28 April 2017; submission 27 December 2016.

Citation
Choi JC, Park YH, Park SK, Lee JS, Kim J, Choi JI, Yoon KB, Lee SE, Lim DE, Choi JY, Kim MH, Park G, Choi SS, Lee JM. Testosterone effects on pain and brain activation patterns. Acta Anaesthesiologica Scandinavica 2017.

doi: 10.1111/aas.12908

Background: This study investigated whether pain and pain-related unpleasantness ratings were altered by blood testosterone levels. We also investigated whether activation of brain regions that represent pain intensity [primary somatosensory cortex (S1)] and pain-related unpleasantness [perigenual ACC (pACC) and orbitofrontal cortex (OFC)] were affected by blood testosterone levels.
Methods: Twenty-six healthy men were recruited. Blood testosterone levels were measured before fMRI scanning. The participants were classified into two groups (high vs. low testosterone) according to their blood testosterone level (each group $n = 13$). The middle finger was immersed in a 50°C water bath (50°C, 30 s, five times) to induce identical noxious stimulation in all participants.
Results: The low testosterone group showed statistically significantly higher pain ($P = 0.047$), unpleasantness ($P = 0.047$), anxiety ($P = 0.015$), and fear ratings ($P = 0.01$) than the high testosterone group. Fear rating increased as pain rating rose and as testosterone level decreased ($P < 0.001$). When participants received noxious stimulation, the pACC and OFC were more highly activated in the low testosterone group compared to the high testosterone group. Activation of S1, a region related to pain intensity, did not differ between both groups.
Conclusion: Compared to the high testosterone group, the low testosterone group had significant activation in the pACC and OFC, regions that represent pain-related unpleasantness, but not in S1 that represents pain intensity, leading to higher pain ratings. These findings emphasize the importance of considering the effects of testosterone levels when treating patients.

Editorial Comment
There are indications from earlier research that testosterone levels can influence pain behavior. In this experimental pain study in male volunteers, pain and pain-related unpleasantness ratings were lower in males with higher testosterone levels, compared to those with lower levels. Brain regions involved in pain affect were also less activated in the high testosterone group.

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Acta Anaesthesiologica Scandinavica
AN INTERNATIONAL JOURNAL OF ANAESTHESIOLOGY AND INTENSIVE CARE, PAIN AND EMERGENCY MEDICINE

Men with low levels of testosterone tend to be more anxious and irritable than men with normal testosterone levels.[1] Low serum testosterone levels in humans have been reported during times of psychological and physical stress, as well as during the acute stress of surgery.[2] Anticipatory stress has been shown to decrease testosterone levels.[3] During periods of stress, testosterone level is positively correlated with pain threshold.[4] Animal studies have also shown that testosterone has anti-anxiety and analgesic effects[5] and modulates opioid analgesia.[6]

Lower levels of serum testosterone in men are associated with subsequent development of rheumatoid arthritis.[7] Lower testosterone levels have been found in both male and female patients with rheumatoid arthritis compared with healthy controls.[8–11] The incidence of rheumatoid arthritis is 4–5 times more frequent in women than men.[7,12] Women have higher pain ratings, lower thresholds, and reduced tolerance to noxious stimuli than men.[13] This indicates that testosterone may be responsible for gender differences in pain sensitivity because on average, testosterone levels in adult men are about 7–10 times higher than those in adult women.[14] In our previous study, pain and unpleasantness ratings were significantly higher in women than in men.[7] Pain perception has been found to increase with decreased testosterone levels,[4,15–18] and brain activation has been shown to correlate with testosterone levels.[19,20] These findings indicate that testosterone levels influence an individual's susceptibility to noxious stimulation, and suggest that low testosterone level may increase nociceptive input and pain ratings by affecting pain-related regions in the central nervous system.

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.[21] Although the sensory and affective components of pain are highly correlated under most circumstances, as pain becomes more intense, it usually becomes more unpleasant.[22] The anterior cingulate cortex (ACC) in humans is the brain region that is most frequently activated by acute nociceptive stimulation.[23,24] In addition, amplification of unpleasantness during noxious stimulation enhances activation of the perigenual ACC (pACC).[24,25] Without changing the perceived intensity, hypnotic suggestions for increased unpleasantness reveal significantly greater activation only in the ACC while S1 activation is unaltered.[26.]

The orbitofrontal cortex (OFC) receives inputs from all sensory modalities.[27] It also receives dense projections from nearly all parts of the ACC.[27] The human OFC is an important brain area involved in the processing of rewards and punishments.[27] In a previous study, although identical noxious stimulation was applied to all participants, the OFC was activated by a relatively higher rank of pain.[28] It has been reported that an affectively unpleasant pain stimulus results in relatively greater activation of the OFC than a neutral stimulus.[29] These previous studies suggest that the OFC may be activated by pain-related unpleasantness (pain affect).

This study investigated whether pain and pain-related unpleasantness ratings are altered by blood testosterone levels. In addition, we investigated whether activation of brain regions that are related to pain intensity and pain-related unpleasantness ratings is affected by blood testosterone levels.

Methods

This study has been performed according to the Declaration of Helsinki. The Medical Ethics Committee of Yonsei University Wonju College of Medicine (Wonju, Kwangwon-Do, South Korea) approved this study (CR313017, 24/9/2013). Twenty-six healthy men were recruited to participate in the pain experiment. A venous blood sample was drawn to determine each participant's testosterone level before the experiment. The participants were classified into two groups (high vs. low testosterone) based on their blood testosterone level (n = 13 in each group).

To induce noxious stimulation, the distal phalange and distal two-thirds of the middle phalange of the left-hand middle finger were immersed in an 850-ml water bath maintained at 50°C. The participants were informed 15 s in advance that a 50°C (noxious) or 35°C (control) stimulation was to begin which was defined as the anticipation period (pre-stimulation or pre-control, respectively). A 30-s finger immersion stimulation at 50°C (noxious) or 35°C (control) stimulation was followed by a 30-s post-

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stimulation or post-control period, respectively. This 168-s block was repeated five times for each participant (Fig. 1). Identical noxious stimuli (50°C, 30 s, five times) were given to all participants.

The participants wore insert earphones connected to a microphone through which they received instructions during each experimental period. Switching between each period was verbally cued. To minimize anxiety level and familiarize participants with finger immersion into the hot bath, each underwent a 30-s finger immersion stimulation at 50°C on the left middle finger before the fMRI scan began. The water temperature was decreased from 50°C to approximately 49.5°C during scanning for the first three blocks of the total five. We designed an interscan resting interval of 5 min between the third and fourth block. During this 5-min interval, the research assistant increased the water temperature from approximately 49.5 to 50°C. A switching period of 3 s (cueing period) between each period was discarded from the fMRI analysis to eliminate effects from middle finger movement.

Ratings during the noxious stimulation were assessed on a numerical rating scale (0 = no pain, unpleasantness, anxiety, and fear; 100 = maximum imaginable pain, unpleasantness, anxiety, and fear). Participants were asked to rate pain, unpleasantness, anxiety, and fear during the 5 min interscan interval between the third and fourth block and at the end of all five blocks. Pain, unpleasantness, anxiety, and fear ratings were averaged for each participant and a group average was calculated.

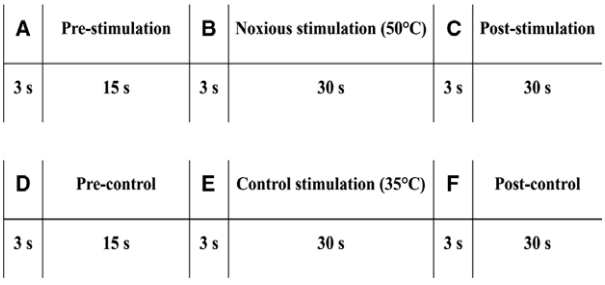

Fig. 1. Study protocol. The following instructions were given to participants. (A) In fifteen seconds, please immerse your middle finger into the hot water bath (50°C). (B) Please immediately immerse your middle finger into the hot water bath (50°C). (C) Please remove your middle finger. (D) In fifteen seconds, please immerse your middle finger into a tepid water bath (35°C). (E) Please immediately immerse your middle finger into a tepid water bath (35°C). (F) Please remove your middle finger. This sequence was repeated five times. A switching period of 3 s (cueing period) between each period was discarded from the fMRI analysis to eliminate the effects from movement of the middle finger.

A venous blood sample was drawn from the right forearm to determine each participant's testosterone level before the experiment. Testosterone levels were measured using the COBRA 5010 Quantum γ-counter (Packard, Meriden, CT, USA) with Coat-A-count Testosterone (Siemens, Los Angeles, CA, USA) kit. The coefficient of variation for testosterone was 5.90%.

The participants were classified into two groups (high vs. low testosterone) according to their blood testosterone level (each group $n = 13$). The high testosterone group comprised those with testosterone levels ranging from 5.6 to 9.5 ng/ml, whereas the low testosterone group comprised testosterone levels ranging from 2.3 to 5.5 ng/ml.

Statistical analyses of testosterone levels and behavioral data

Group data were analyzed using an independent samples *t*-test with PASW (Predictive Analytics Software) Statistics version 20 (SPSS Inc., Chicago, IL, USA). Data from all 26 participants were included in regression analysis, with testosterone analyzed as a continuous variable. $P < 0.05$ was considered statistically significant.

Functional imaging

Before the scan, the participants were instructed to keep their eyes closed, to stay awake, and to refrain as much as possible from moving throughout the imaging session. After being placed in a comfortable position, the head was immobilized with padded ear muffs and a foam headrest, and a plastic bar was placed across the bridge of the nose. Images were acquired using a KASIT 3-T MRI scanner (ISOL Technology, Gwangju, Kyonggi-Do, Korea) with a quadrature head coil. After a T1-weighted scout image, high-resolution anatomic images were acquired using a magnetization-prepared rapid gradient echo pulse sequence with time to echo (TE) = 16 ms, repetition time (TR) = 2800 ms, flip angle = 60°, and matrix size = 192 × 256 mm. The T2*-weighted

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functional data were acquired using an echo planar imaging pulse sequence of TE = 30 ms, TR = 3000 ms, flip angle = 80°, and matrix size = 64 × 64 mm. Twenty-eight slices of the echo planar images were obtained with a 5 mm slice thickness. The experiment consisted of two experimental runs: the first run consisted of three blocks, whereas the second run consisted of two blocks. The initial five images from each scan were discarded, and all images scanned during the cueing period (3 s) were also discarded.

Statistical analyses of fMRI data

Preprocessing of the Task-State fMRI (TS fMRI) data was performed using Analysis of Functional NeuroImage (AFNI) software.[30] To correct for slice timing errors and head motion errors during the TS echo-planer imaging (EPI) time courses, 3dTshift and 3dvolreg commands in AFNI were applied.[31] Spatial smoothing was then performed on each image using an 8-mm full width at half maximum Gaussian kernel. We had to estimate an impulse response function and multilinear regression analysis for the time series data. The impulse response function was convolved with the stimulus time series to yield the estimated response.[32] The estimated impulse response function and multilinear regression model were processed using 3dDeconvolve in AFNI. Images were then normalized to a standard MNI152 template and resampled at an isotropic voxel size of 2 mm using the AFNI @auto_tlrc command.

A two-sample *t*-test was performed to compare the two groups (low testosterone vs. high testosterone). We obtained a corrected significance level of $P_\alpha < 0.05$ (uncorrected threshold of $P_\alpha < 0.001$ with a cluster size of 77 voxels) by using the 3dClustSim program from AFNI. The 3dClustSim program provides an estimate of the overall significance level achieved for various combinations of individual voxel probability thresholds and cluster size thresholds.[33] Monte Carlo simulation with the 3dClustSim program was simulated 10,000 times iteratively, with an 8-mm FWHM Gaussian filter width in a whole-brain mask for type I error control.

The post-central cortex (S1) is the region that represents brain responses to pain intensity. To test brain activation to noxious stimulation in the post-central cortex, a one-sample *t*-test was analyzed for both high and low testosterone groups. We observed that almost the entire brain was significant at the threshold (corrected significance level of $P_\alpha < 0.05$, uncorrected threshold of $P < 0.001$ with a cluster size of 77 voxels) for the two-sample *t*-test. Therefore, the thresholds of the one-sample *t*-test were modified to show activation of the post-central cortex in both groups.

Results

Behavioral and hormonal data

Age, height, and weight were not different between participants in the high and low testosterone groups (Table 1). Testosterone levels were significantly higher in the high testosterone group compared to the low testosterone group ($P = 0.047$). The participants with low testosterone levels showed significantly higher pain and pain-related unpleasantness ratings than those with high testosterone levels ($P = 0.047$) (Table 1). Moreover, the anxiety ($P = 0.015$) and fear ($P = 0.001$) ratings during the noxious stimulation were statistically significantly higher in the low testosterone group than in the high testosterone group (Table 1).

Regression analysis included data from all 26 participants. The regression equation for fear rating was as follows: fear rating (Y) = 14.75 + 0.64 (pain rating) − 4.43 (testosterone level). This model was statistically significant (adjusted $R^2 = 0.59$, $F = 19.13$, $P < 0.001$). Pain rating ($P < 0.001$) and testosterone level ($P = 0.01$) contributed significantly to fear rating. These results indicate that fear rating increased as pain rating rose and as testosterone level decreased.

fMRI data

Two-sample t-*test*

With the threshold selected in this study (corrected significance level of $P_\alpha < 0.05$, uncorrected threshold of $P < 0.001$ with a cluster size of 77 voxels), the pACC, OFC, and cuneus were more highly activated in the low testosterone group compared to the high testosterone group when participants received identical noxious

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Table 1 Comparison of participant characteristics, testosterone level, and self-reported measures between the high and low testosterone groups.

	High testosterone group (n = 13)	Low testosterone group (n = 13)	P value
Age (years)	23.08 ± 2.33	22.15 ± 2.41	0.330
Height (cm)	177.48 ± 7.95	172.62 ± 8.18	0.137
Weight (kg)	72.46 ± 11.18	69.62 ± 8.03	0.463
Testosterone level (ng/ml)	6.65 ± 1.13	4.32 ± 0.96	< 0.001
Pain rating	39.85 ± 23.33	55.85 ± 14.56	0.047
Pain-related unpleasantness rating	29.69 ± 21.05	45.00 ± 15.91	0.047
Anxiety rating	22.31 ± 23.06	42.70 ± 15.90	0.015
Fear rating	9.23 ± 12.56	33.08 ± 17.97	0.001

The data shown are the mean ± SD. The data were analyzed using an independent samples t-test. The ratings were assessed on a numerical rating scale (0 = no pain, unpleasantness, anxiety, and fear; 100 = maximum imaginable pain, unpleasantness, anxiety, and fear).

Table 2 Brain areas more highly activated in the low testosterone group compared to the high testosterone group.

	Coordinates	Voxels	Maximum t-values
Rt cuneus	20, −96, 18	593	5.91
Rt perigenual ACC	2, 42, 14	136	4.84
Lt OFC	−2, 32, −22	114	5.97

When participants received identical noxious stimulation, the perigenual anterior cingulate cortex (pACC), orbitofrontal cortex (OFC), and cuneus were more highly activated in the low testosterone group compared to the high testosterone group (corrected significance level of $P_\alpha < 0.05$, uncorrected threshold of $P < 0.001$ with a cluster size of 77 voxels). Rt = Right. Lt = Left.

stimulation (Table 2 and Fig. 2). With this threshold, no brain regions were more highly activated in the high testosterone group compared to the low testosterone group when participants received noxious stimulation. Activation of the primary somatosensory cortex (S1) classically associated with nociception did not differ between the two groups.

One-sample t-*test*

Bilateral post-central cortices (S1) in the low testosterone group were activated with a P value of 1*e-14 [t-value and number of voxels were 17.63 and 29, respectively, in the left S1 (−32, −36, 68), t-value and number of voxels were 28.16 and 20, respectively, in the right S1 (66, −14, 20)]. The left post-central cortex (S1) in the high testosterone group was activated with a P value of 1*e-12 [t-value and number of voxels were 22.78 and 113, respectively, in the left S1 (−36, −40, 64)].

Discussion

In this study, participants with lower testosterone levels showed significantly higher pain and pain-related unpleasantness ratings than those with high testosterone levels. Also, anxiety and fear ratings were statistically significantly higher in the low testosterone group than in the high testosterone group. The pACC and OFC, regions that represent brain responses to pain-related unpleasantness, were more significantly activated in the low testosterone group compared to the high testosterone group.

In our previous study with humans,[16] testosterone levels were significantly negatively correlated with average pain rating. Across both men and women participants, as testosterone level increased, functional connectivity between the periaqueductal gray (PAG) area and lateral prefrontal cortex (PFC) significantly increased for the pain+TENS compared to the pain-only condition. [In the pain+TENS condition, noxious stimulation and transcutaneous electrical nerve stimulation (TENS) is concurrently presented. In the pain-only condition, noxious stimuli were presented without TENS.] This previous study suggested that testosterone and TENS might lead to activation of the descending pain inhibitory pathway, resulting in decreased pain perception. The lateral PFC and PAG have been implicated in endogenous pain inhibition.[34] In the threshold used for statistical significance in this study, although no brain regions were activated in the high testosterone group compared to the low testosterone group, the pACC and OFC were significantly more activated in the low testosterone group compared to the high testosterone group. This indicated that pain-related unpleasantness and pain perception might be increased in the low testosterone group when the pACC and OFC were activated.

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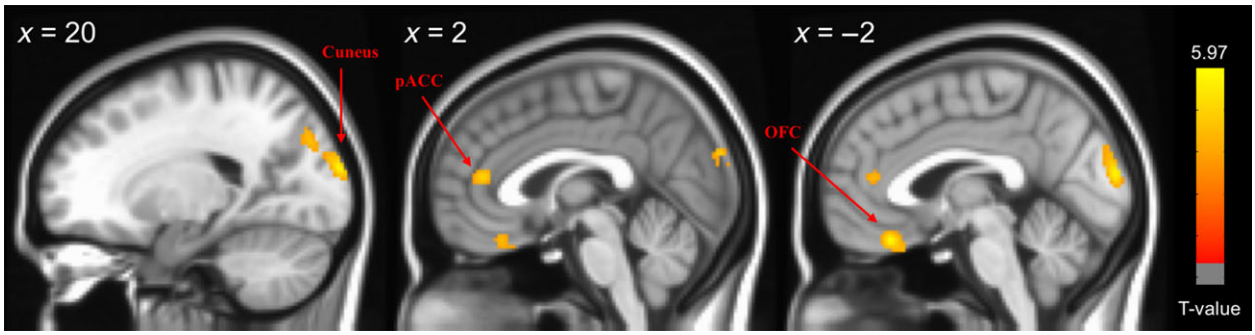

Fig. 2. Brain areas more highly activated in the low testosterone group compared to the high testosterone group. When participants received identical noxious stimulation, the right perigenual anterior cingulate cortex (pACC), left orbitofrontal cortex (OFC), and left cuneus were more highly activated in the low testosterone group compared to the high testosterone group (corrected significance level of $P_{\alpha} < 0.05$, uncorrected threshold of $P < 0.001$ with a cluster size of 77 voxels).

Although all participants received identical noxious stimulation, the pACC was more significantly activated in the low testosterone group compared to the high testosterone group. Negative affect is related to activation of the pACC and midcingulate cortex, whereas the midcingulate cortex is related to brain responses to noxious stimulation.[35] Attention to unpleasantness increases responses in bilateral pACC after noxious laser stimuli.[25] As pain-related unpleasantness is processed in the pACC,[24,25] activation of the pACC suggests that participants with low testosterone levels significantly perceived more pain-related unpleasantness, and therefore may have higher activation in the pACC compared to those with high testosterone levels.

Although identical noxious stimulation was applied to all participants, the OFC was more significantly activated in the low testosterone group compared to the high testosterone group. This finding is supported by a recent pain experiment from Winston et al.[28] In their experimental design, three pain levels were used for stimulation (low, medium, and high), and were arranged in blocks such that medium pain could be surrounded by either low pain or high pain. Critically, medium pain therefore changed its (current) relative rank between these different blocks – in the blocks when it was paired with high pain, it represented the lowest ranked pain that was currently experienced, whereas in the blocks when it was paired with low pain, it represented the highest ranked pain that was currently experienced. The OFC had a completely relative response to pain so that when medium pain was surrounded by low pain, the response to medium looked like the response to high, and when medium pain was surrounded by high pain, the response to medium looked like the response to low. The OFC was activated by the relative (current) high pain rank, even though identical noxious stimulation was used for medium pain within the two different contexts. In this study, although participants in both groups received identical noxious stimulation, participants with low testosterone levels reported high unpleasantness ratings, and therefore may have had more significant activation in the OFC compared to those with high testosterone levels.

Activation of S1 classically associated with nociception in both groups did not differ in a two-sample *t*-test, although S1 in both groups was activated in a one-sample *t*-test. Because the primary somatosensory cortex (S1) contains neurons activated by noxious somatosensory stimuli, S1 discriminates noxious somatosensory stimuli.[34,36] Because participants in both groups received identical noxious stimulation (50°C, 30 s, five times), S1 that represents pain intensity might not be different between groups.

Activation of the pACC and OFC, regions that represent brain responses to pain affect, suggested that participants with low testosterone levels perceived significantly more pain-related unpleasantness than those with high testosterone levels. Although all participants received identical noxious stimulation, as the pACC and OFC were more highly activated in participants with low testosterone levels compared to those

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with high testosterone levels, participants with low testosterone levels may perceive more pain-related unpleasantness and pain intensity than those with high testosterone levels. In other words, an increase in pain-related unpleasantness in participants with low testosterone levels may lead to an increase in pain intensity (pain ratings) by activating the pACC and OFC.

Conclusion

These results indicated that pain and pain-related unpleasantness ratings were statistically significantly lower in participants with high testosterone levels than in those with low testosterone levels. The pACC and OFC, regions that represent brain responses to pain affect, were more highly activated in the low testosterone group compared to the high testosterone group. These findings emphasize the importance of considering the effects of testosterone level when designing clinical studies or when treating patients for pain and pain-related unpleasantness.

Acknowledgment

The authors thank Joel Winston MRCP PhD (Wellcome Trust Centre for Neuroimaging, UCL, London) for fruitful discussions and valuable suggestions.

Author's contributions

J. C. C.: designed the study.

J. C. C. and S. K. P.: collected data.

J. C. C., S. S. C., J. I. C., K. B. Y., S. L., D. E. L., J. Y. C., M. H. K., and G. P.: conducted the behavioral data analysis.

J.-M. L., Y.-H. P., J. S. L., and J. K.: conducted the fMRI data analysis.

J. C. C.: wrote all of the manuscript except for the fMRI data analysis section in the methods.

Y.-H. P. and J. S. L.: wrote the fMRI data analysis section in the methods.

All authors read and approved the final manuscript.

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