INTRODUCTION:
The observation that an experimental pinealectomy in newborn chickens leads to a spinal deformity similar to idiopathic scoliosis in humans initiated a new neuroendocrine hypothesis for the cause of idiopathic scoliosis. A deficiency of melatonin, which is the principal product of the pineal gland, was believed to be responsible for this deformity, because both autografting of the pineal gland and substitution of melatonin prevented the development of scoliosis in the pinealectomised chickens. Machida et al (1) also reported significantly lower levels of serum melatonin in adolescents with progressive scoliosis compared with patients with stable scoliosis or healthy control subjects. However, no alteration in the serum or urinary melatonin level in patients with adolescent idiopathic scoliosis was found by others. Therefore, the role of the pineal gland function in the pathogenesis of human adolescent idiopathic scoliosis remains unknown.

We examined the urinary excretion of 6-sulfatoxyl-melatonin and the pineal gland glucose metabolism using F-18 fluorodeoxyglucose (FDG) brain positron emission tomography (PET) in patients with adolescent idiopathic scoliosis and in gender-matched healthy control subjects.

METHODS:
We studied fourteen adolescent patients who had been admitted to our department for the surgical correction of scoliosis. The Cobb angles of the curves were measured on plain radiographs and the Risser signs were determined. Ethical approval of this study was obtained from the Research Ethics Committee of the Pusan National University Hospital. All patients aged between 11 and 14 years presenting to the adolescent idiopathic scoliosis were asked to voluntary provide urine samples over a 24-hour period for measurement of 6-sulfatoxyl-melatonin excretion and to examine pineal gland metabolism. Thirteen gender-matched young adults were used as the control group. All the control subjects had a straight spine and a normal forward bending test on the physical examination with no history of spinal disease. Written consent was obtained from the participants and their parents. No monetary compensation was given to the control group.

Because melatonin secretion occurs in a diurnal rhythm with nocturnal peak, the 24-hour collection was divided into four periods of 6 hours. These samples were obtained from each patient and control subject between 7 am and 7 pm and 7 pm and 7 am. Total urine volumes for each of the 12–hour periods were determined before an 10 ml aliquot was stored at -20°C for measurement of the 6-sulfatoxyl-melatonin concentration. To avoid the nocturnal suppression of melatonin, light was restricted to less than 200 lux from 8 pm to 7 am. In addition, to avoid the possible influence of stress on the level of melatonin secretion in the patients, urine was collected at least one day before surgery. At the completion of the study period, the urinary 6-sulfatoxyl-melatonin concentration was determined by a direct radioimmunoassay (Stockgrand, Guildford, UK) on all samples, from which the diurnal excretion of 6-sulfatoxyl-melatonin was calculated.

Brain MRI. MR imaging studies were performed using a 1.5-T superconducting magnet and a standard head coil (Sonata™, Siemens, Erlangen, Germany). A 5-mm section thickness, 1.8-mm intersection gap, and a 200 x 200-mm field of view were used for all sequences.

F-18 FDG Brain PET. PET scans of a single frame of 15 min were acquired beginning 60 min after the intravenous injection of 4.8 MBq/kg F-18-FDG using a Gemini PET/CT scanner (Gemini™, Philips, Milpitas, CA, USA). The scans were performed on the subjects in the resting condition with their eyes closed and ears unplugged, and lying comfortably in a darkened and quiet room. The subjects fasted for at least 6 hours before PET imaging. The PET images were reconstructed using a 3-dimensional row action maximum likelihood algorithm (3D RAMLA) (2 repetition, 0.006 relaxation parameter) and were displayed in a 128 x 128 matrix (pixel size = 2 x 2 mm, with a slice thickness of 2 mm). Attenuation correction was performed with a uniform attenuation coefficient (μ = 0.096 cm⁻¹). The in-plane and axial resolution of the scanner were 4.2 and 5.6 mm full width at half maximum (FWHM), respectively.

Semi-quantitative analysis of F-18 FDG brain PET. A brain MRI was reoriented three-dimensionally to the anterior to posterior commissure line and an exact 3-dimensional alignment of the brain PET scan with the MRI was performed using a previously described coregistration method. The pineal gland was identified on the MRI image, and a circular region of interest (ROI) with 8 mm in diameter was placed under visual control of the transverse image on the MRI, where the maximum size of the pineal gland was visible. After placing a rectangular reference ROI (30 x 30 mm) over the cerebellum on the transverse plane, both ROIs were transferred to the coregistered F-18 FDG brain PET image. We assessed the mean relative values of the cumulative tracer activity within both ROIs on the coregistered image. The relative tracer activity in the pineal gland in comparison with the cerebellar reference (P/Cbl) was calculated using the following formula; P/Cbl = pineal gland ROI activity/cerebellar ROI activity.

Statistical parametric mapping (SPM) analysis of F-18 FDG brain PET. Spatial preprocessing and statistical analysis were performed using the SPM2 implemented in Matlab 5.3 (The MathWorks, Inc., Natick, MA). All the reconstructed F-18-FDG PET images were spatially normalized into Montreal Neurological Institute (MNI, McGill University, Montreal, Que., Canada) standard templates using an affine transformation (12 parameters for rigid transformations) and a non-linear transformation. The images were then smoothed with a FWHM 8-mm Gaussian kernel to increase the signal-to-noise ratio and to account for subtle variations in anatomic structure. The count of voxel was normalized to the average count of the cerebellum using a customized program, because the cerebellum is known to be one of the least affected regions. Images of the scoliosis patients were compared with those of the healthy normal controls in a voxel-wise manner using SPM2 for the between-group analysis (P < 0.001, uncorrected, extent threshold, k = 10). For the group analysis, a 2-sample t test was used to detect differences between the scoliosis and healthy control group. The Talairach brain coordinates were estimated from a non-linear transformation from MNI space to Talairach space (Talairach Daemon Client, Version 1.1, Research Imaging Center, University of Texas Health Science Center at San Antonio). The differences between the scoliosis and the healthy control group were examined using an extent threshold of 10 voxels with a p value <0.001 to illustrate the group differences in the statistical voxel-based analysis, as well as for illustrating the result of the registration between the scoliosis and the healthy control group.

RESULTS:
The mean diurnal and nocturnal concentration of 6-sulfatoxyly-melatonin in the patients were 1.4 ng/ml (0.6 to 2.8) and 7.9 ng/ml (3.3 to 16.2), respectively, whereas, the median was 1.2 mg/ml (0.5 to 2.3) and 6.5 mg/ml (1.8 to 12.3) in the control group, respectively. There was no significant difference in 6-sulfatoxyly-melatonin excretion or any of its transformations between the scoliosis patients and the control subjects. The maximum pineal gland metabolism (maximal P/Cbl) of the patients and control subjects was a median of 0.635 (95% CI, 0.5324 to 0.7711) and 0.580 (95% CI. 0.5555 to 0.634), respectively. The mean relative tracer activity in the pineal gland metabolism (mean P/Cbl) of the patients and control subjects was a median of 0.565 (95% CI, 0.5247 to 0.6100) and 0.54 (95% CI, 0.4700 to 0.5711), respectively. The Mann-Whitney U test of the pineal gland metabolism showed no statistically significant differences between the patients and the control group (Maximal, p = 0.4667; Mean, p = 0.0726). There was no statistically significant correlation between the pineal gland metabolism and the mean diurnal and nocturnal concentration of 6-sulfatoxyly-melatonin in the study subjects.

The objective voxel based analytic method of SPM showed no statistically significant hypometabolic lesion of the brain including the pineal gland in the scoliosis patients compared with the normal healthy controls.

CONCLUSION:
Although the morphology of scoliosis in melatonin deficient chickens closely resembles human scoliosis and despite the constant reproducibility of this effect in newborn chickens, the effect of melatonin and the pineal gland metabolism on human adolescent idiopathic scoliosis is still unclear. Permanent melatonin deficiency and abnormal pineal gland metabolism was not observed in patients with adolescent idiopathic scoliosis.

REFERENCES: