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MON-715

Many transcription factors have been identified that are important for specification and differentiation of the hormone-producing cells of the pituitary gland. For example, POU1F1 is essential for differentiation of thyrotropes, somatotropes, and lactotropes. GATA2 is important for differentiation of both thyrotropes and gonadotropes, and NR5A1 drives gonadotrope differentiation. It is not known whether additional transcription factors are involved in thyrotrope specification. A few POU1F1-independent thyrotropes arise during development at the rostral tip of the pituitary gland, but the majority of thyrotropes are POU1F1-dependent and arise in the caudo-medial area of the anterior pituitary gland. Pou1f1-deficient mice lack this major, critical type of thyrotropes and are severely hypothyroid. Several pieces of evidence suggest that the caudo-medial population of thyrotropes is heterogeneous. First, NR5A1-positive cells exist that express both TSH and FSH during pituitary development. Second, Pou1f1 mRNA is detected in only about half of pituitary cells immuno-positive for TSH. Third, the transcription factor ISL1 is only detected in a fraction of developing thyrotropes. The Helix-Loop-Helix transcription factor ASCL1 is critical for zebrafish pituitary development, but there are conflicting data about its role in pituitary thyrotrope specification in mice. To quantify the role of ASCL1 in thyrotrope development we carried out immunostaining for TSH in pituitaries of Ascl1 deficient mice in late gestation. We did not detect any reduction in TSH immuno-reactive cells at this time, suggesting there are species-specific differences in the requirement of ASCL1 in the developing pituitary. To systematically assess and quantify the heterogeneity of thyrotropes for transcription factor expression, we performed co-immunostaining for TSH and various transcription factors in pituitaries of newborn mice. We found that approximately 75% of TSH-positive cells in the caudo-medial region of the pituitary express POU1F1, and the majority express GATA2. We detected a small portion of cells co-expressing NR5A1 and TSH at this time. To confirm our immunostaining results, we analyzed data from single cell RNA sequencing of postnatal thyrotropes. Three robust clusters of thyrotropes were identified. Approximately 80% expressed Gata2, 85% expressed Pou1f1, and roughly 15% expressed Nr5a1. Taken together, the protein and RNA analysis support that thyrotrope heterogeneity exists during development. The functional significance of this heterogeneity and its variation during development will be the subject of future investigation.

Bone and Mineral Metabolism

BONE DISEASE FROM BENCH TO BEDSIDE

Associations of Trabecular Bone Score and Bone Mineral Density with Cardiorespiratory Fitness and Body Composition in Men with and Without Paraplegia

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SUN-355

Associations of Trabecular Bone Score and Bone Mineral Density with Cardiorespiratory Fitness and Body Composition in Men with and without Paraplegia

Introduction: Following spinal cord injury (SCI) lower extremity bone mineral density (BMD) losses are as high as 40% due to mechanical unloading and autonomic dysfunction. While lumbar spine (LS) BMD appears to be relatively spared, evidence suggests BMD by dual-energy radiographic absorptiometry (DXA) may overlook bone pathology in this region. Trabecular Bone Score (TBS), representative of bone microarchitecture, may be a more informative measurement of LS pathology in SCI. Our objective was to determine differences in BMD and TBS within the LS in humans with and without SCI. Correlation of fitness and body composition measures with TBS and BMD were also explored to determine their role in bone health after SCI.

Methods: Seven male participants with paraplegia (level T3 – T7) were recruited through The Miami Project to Cure Paralysis, and 6 males without SCI were recruited from local advertisement. DXA scans of the lumbar spine and whole body were performed using Hologic Discovery A densitometer. TBS score (unitless) was derived from L1-L4 scans using TBS iNsight software v3.0.2. A graded arm exercise test directly measured cardiorespiratory fitness (VO_2peak) for all subjects. An independent samples t-test determined between-group differences in LS BMD and TBS. Pearson correlation analysis investigated within-group relations among LS BMD, TBS and VO_2peak (ml/kg/min), weight (kg), total body fat (%), and visceral adipose tissue (estimated VAT mass(g)).

Results: In SCI, the mean duration of injury was 8.6 years. Mean LS BMD was not different (p=.47) between non-injured (1.10 ±0.11 g/cm^2) and SCI (1.10 ±0.13 g/cm^2) groups. However, mean TBS score was different (p=.053) between non-injured (1.55±0.09) and SCI (1.47±0.07) groups. In non-injured, VO_2peak was correlated with LS BMD (r=.356) and TBS (r=.244). In SCI, VO_2peak was correlated with LS BMD (r=.111) and TBS (r=.822). In non-injured, TBS was correlated with body mass (r=.244), total body fat (%) (r=.382), and visceral adipose tissue (r=.361). In SCI, negative correlations were observed; TBS was correlated with body mass (r= -.255), total body fat (%) (r= -.474), and visceral adipose tissue (r= -.513). LS BMD was positively correlated with body mass, total body fat (%) and visceral adipose tissue in both non-injured and SCI groups.

Conclusion: Men with and without SCI displayed similar BMD but differed in TBS at the LS. Correlations with measures of fitness and body composition were similar for LS BMD but discordant for TBS between non-injured and SCI groups. The data suggest changes in the relationships between cardiorespiratory fitness, metabolism and bone quality in SCI. TBS may capture alterations in bone microarchitecture at the spine after SCI that are undetected by conventional DXA.

Reproductive Endocrinology

FEMALE REPRODUCTION: BASIC MECHANISMS

Progesterone Receptor Expression in Endometrial Biopsies After 12 Weeks of Exposure to A 4-µg E2 Softgel Vaginal Insert or Placebo

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The softgel, vaginal 17β-estradiol (E2) insert (TX-004HR) significantly improved the maturation index of the vaginal epithelium, dyspareunia and vaginal dryness in menopausal women with vulvar and vaginal atrophy (VVA) and moderate to severe dyspareunia, without histological changes to the endometrium (Constantine G et al, *Menopause* 2017;24:409-416). The 4-µg and 10-µg E2 doses were FDA approved as Imvexxy® (TherapeuticsMD, Boca Raton, FL), for the treatment of moderate to severe dyspareunia, a symptom of VVA, due to menopause. The progesterone receptor (PR) is an estrogen-regulated gene with expression that is very sensitive to E2 exposure (Xiao CW and Goff AK, *J Reprod Fertil*.1999;115:101-109). Endometrial PR expression in the biopsies of women using the softgel vaginal 4-µg E2 insert was used as a marker of E2 exposure to determine whether sufficient E2 applied with the vaginal insert reaches the endometrium to upregulate PR expression. Our hypothesis posits that there would be insufficient E2 from the 4-µg E2 insert to stimulate an increase in endometrial PR expression.

In this post hoc analysis of the REJOICE trial, endometrial biopsies from 25 women who had a normal baseline biopsy, an on-therapy biopsy after study day 70, and tissue readings from all pathologists were randomly selected from the 4-µg E2 vaginal insert and placebo groups. Endometrial tissue sections were stained to visualize PR expression using PgR1294 (Agilent; Santa Clara, CA). Cell staining was quantified using a trainable feature-recognition algorithm and mean expression levels between baseline and week 12 were analyzed by 2-sided t-tests.

Acceptable PR expression results were available for 22 women in the 4-µg E2 group (three had insufficient tissue for analysis) and 25 women in the placebo group. For the 4-µg E2 group, mean ± SD (pmol/mg) PR expression was 0.455 ± 0.203 at baseline and 0.506 ± 0.226 at week 12. For the placebo group, mean PR expression was 0.579 ± 0.196 at baseline and 0.563 ± 0.213 at week 12. Mean PR expression levels at baseline and week 12 were not significantly different from each other within the 4-µg E2 (P=0.438) or placebo (P=0.783) group. No meaningful difference in endometrial PR expression was observed with the vaginal 4-µg E2 insert at week 12. These data support our hypothesis and the assertion that low-dose, local vaginal E2 exposure with the insert placed in the lower part of the vagina, does not pose an endometrial safety issue in postmenopausal women.

Adrenal

ADRENAL PHYSIOLOGY AND DISEASE

The Intra-Individual Variability of 11-Ketotestosterone and 11β-Hydroxyandrostenedione.

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SUN-208

Background

Emerging evidence has suggested the 11-oxygenated steroids may be important in the diagnosis and monitoring of hyperandrogenism. Two in particular, 11-ketotestosterone (11KT) and 11β-hydroxyandrostenedione (11OHA4) have been implicated in polycystic ovary syndrome, congenital adrenal hyperplasia, precocious puberty and castration resistant prostate cancer. Despite the interest in these analytes, some of their more fundamental properties have yet to be determined. At present, no data is available that quantifies the biological variation of 11KT and 11OHA4 within individuals over time, this may be important as we look to establish normative reference ranges for these potentially useful analytes.

Objective

Here we sought to define the intra-individual variability of 11KT and 11OHA4 in serum using LC-MS/MS.

Method

Blood was collected from 18 healthy volunteers (8 males, 10 females) on the same day each week over a 10 week period using standard venepuncture technique. After collection, the samples were centrifuged within 1 hour, aliquoted and stored at -20°C (-4°F) prior to analysis. All samples from individual volunteers were analysed by LC-MS/MS in triplicate within the same batch so as to limit analytical variability.

Results

The mean analytical coefficient of variation (CV%) for the triplicate analysis was 3.2% for 11KT and 3.7% for 11OHA4. No significant difference was observed between the variability of 11KT concentrations in the male and female cohorts; total intra-individual variation for 11KT was 18.0%. Concentrations of 11OHA4 were more variable in the male cohort when compared to the female cohort. This was reflected by differences in their respective intra-individual variations of 32.5% vs 24.8%.

Summary

Intra-individual variation is an important consideration when interpreting patient results. Concentrations of 11KT were tightly regulated in both the male and female cohorts with no clear demarcation between the two groups. Although concentrations of 11OHA4 were prone to greater variation over the 10 week period, considerable overlap was observed between the male and female subjects. Our data suggests that 11KT and 11OHA4 concentrations are not significantly affected by the menstrual cycle.

Neuroendocrinology and Pituitary

HYPOTHALAMIC-PITUITARY DEVELOPMENT AND FUNCTION

Pituitary Developmental Defects Caused by Haploinsufficiency for the Transcription Factor SIX3 Are Worsened by POU1F1 Deficiency.

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SAT-288

Advances in genomic technologies are revolutionizing the practice of medicine by delivering molecular diagnoses that can be informative for prognosis and treatment of genetic