by overtraining syndrome (OTS) were compared to a two control groups, of healthy athletes (ATL) and healthy non-physically active controls (NPAC). Since none of the parameters were directly dependent on exercise or performance, differences between these two groups were unexpected. From the fact that several parameters were shown to be different between ATL and NPAC, we realized that the use of the reference ranges for general population to analyze results in athletes may potentially under- and over-diagnose a wide range of conditions. Our objective is therefore to determine whether athletes should be biochemically evaluated through specific adapted ranges, and propose preliminary adaptations in these ranges. **Methods:** A systematic review on the literature on endocrine and metabolic adaptations to exercise was performed, as well as a thorough analysis of the seven arms of the Endocrine and Metabolic Responses on Overtraining Syndrome (EROS) study. **Results:** Multiple reference ranges were shown to be inaccurate for athletes. Among the parameters that should be adapted for athletes, and their respective adapted ranges include: 1. Cortisol response to an insulin stimulation test (ITT) (> 20.5 μg/dL); 2. GH response to an ITT (> 12 μg/L); 3. Prolactin response to an ITT (> 22 ng/mL); 4. Salivary cortisol at 8AM (> 450 ng/dL); 5. Total testosterone (> 450 ng/dL); 6. Estradiol (25-45 pg/mL) - and testosterone-to-estradiol ratio maintained > 13.7; 7. Total nocturnal urinary catecholamines (> 220 μg/12h); 8. Resting lactate (< 1.0 mM/L); 9. Measured-to-predicted basal metabolic rate (BMR) (> 105%); 10. Fat oxidation (in relation to total BMR) (> 50%); and 11. Hydration status (body water > 62% of total body weight). **Conclusion:** Analysis of biochemical parameters in athletes should be interpreted with caution, particularly hormonal and metabolic parameters, once many parameters likely undergo adaptive changes when under physical activity. Preliminary adaptations for the ranges have been proposed.

**Adrenal**

**ADRENAL PHYSIOLOGY AND DISEASE**

**Chronic Cortisol Works Through the Transcription Factor KLF9 to Deregulate Immune Response and Metabolism**

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**SUN-LB40**

Chronically elevated levels of glucocorticoids (GC) are associated with a number of disease states and negative side effects, including metabolic syndrome. Epidemiological studies show that elevated GC during a brief but vulnerable developmental window can have life-long and potentially multi-generational impacts on health. To elucidate underlying pathogenic mechanisms, our lab has used chronic treatment with a physiological dosage of cortisol (CORT) in developing zebrafish, Danio rerio, a model organism that has emerged as a useful tool for investigating GC signaling. In this paradigm, we have found evidence that high CORT during development alters a set point for the HPA axis and leads to continuous induction of aberrant GC production and transport, accompanied by altered immune gene regulation and decreased ability to maintain blood glucose homeostasis. To identify molecular and genetic pathways perturbed by chronic CORT treatment, we used CRISPR to generate mutant lines lacking the glucocorticoid receptor (GR) or the transcription factor Klf9, which we have found to be an important target/regulator of GC signaling. We performed RNA sequencing in these mutant lines and compared the transcriptomes of wild type (WT) and mutant animals treated with either chronic CORT or vehicle control (VEH). A broad overview of the data shows similarities between CORT treated wild-type fish and VEH treated GR mutants suggestive of GC resistance in the CORT treated WT animals. In Klf9 mutants, a number of genes involved in immune processes that were upregulated by chronic CORT in WT animals were not similarly upregulated, suggesting that Klf9 is an important feed-forward mediator of immune gene regulation by GC. Additionally, CORT increased expression of a number of metabolic genes in Klf9 mutants that were not similarly upregulated in WT, suggesting that Klf9 plays a regulatory role in the response of cellular metabolism to GC. To further investigate Klf9’s role in governing cellular metabolism, metabolic rate assays were performed on live animals. The results show that Klf9 mutants have lower total respiration, and that chronic CORT increases non-mitochondrial respiration in both WT and Klf9 mutants. Mitochondrial respiratory capacity was unaffected across conditions. This, coupled with gene expression data, suggests that measured metabolic differences are due to shifts in substrate usage and differential reliance on non-mitochondrial metabolic pathways such as glycolysis and peroxisomal beta-oxidation. Additional studies are required, but the regulation of glycolysis by Klf9 could contribute to this gene’s known tumor-suppressive role, and regulation of peroxisomal metabolism—key in immune cells—could partially explain the role of Klf9 in mediating these cells’ responsiveness to CORT.

**Tumor Biology**

**TUMOR BIOLOGY: DIAGNOSTICS, THERAPIES, ENDOCRINE NEOPLASIAS, AND HORMONE DEPENDENT TUMORS**

**Interleukin-8 - a Possible Target for Melanoma Treatment? In-Vitro Studies Based on Human Melanoma Cell Models**

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**SUN-LB27**

Previous clinical studies showed that menstruating females were better protected in melanoma than post-menopausal women and men of any age. In addition, epidemiological studies showed an increased male mortality in melanoma. But these studies did not correlate with steroid status in females. Our in-vitro study showed female sex hormone progesterone significantly inhibited human melanoma cell growth. Further in-vitro study showed that progesterone action was mediated by a specific suppression...
of pro-inflammatory cytokine IL-8. Our research also showed that addition of IL-8 (1 ng/ml) to melanoma cells stimulated cell growth (117%) and suppression of IL-8 by curcumin (100 μM) pre-treatment suppressed human melanoma cell growth (26%) in-vitro. This observation prompted us to check the effect of male sex hormones androstenedione (AD) and testosterone (T) on melanoma cell growth. AD and T also suppressed cell growth and IL-8 secretion, but not as significantly as that of progesterone. However, addition of progesterone (10 μM) along with androgens showed an additive effect on the inhibition of melanoma cell growth and suppression of IL-8 secretion. As steroids (P, AD, T) targeted IL-8 for their action, it was decided to check whether vitamin-D3 also targeted IL-8 secretion and cell growth. Active form of vit-D3 (25 μM) also suppressed IL-8 secretion and cell growth. But, addition of progesterone (50 μM) along with D3 significantly suppressed cell growth and IL-8 secretion. This brought IL-8 into focus as a key molecule regulating melanoma cell growth. In order to check whether IL-8 was the molecule involved in regulating melanoma cell growth, IL-8 rescue experiment after curcumin (25 μM) pre-treatment was carried out. IL-8 (100 ng/ml) was able to rescue cell growth completely after pre-treatment with curcumin, suggesting IL-8 was the molecule involved in regulating melanoma cell growth. Literature also suggested an important role for IL-8 in regulating melanoma cell growth. Conditional expression of IL-8 in nude mouse by Dr. Singh et al., indicated in-vivo role of IL-8 in melanoma growth and metastasis.

Conclusion: Both, in-vitro and in-vivo studies suggested an important role for IL-8 in regulating melanoma growth and metastasis. So, IL-8 could be targeted to arrest melanoma growth and metastasis in-vivo. Hence, IL-8 could be a potential target for melanoma treatment.

Reproductive Endocrinology

FEMALE REPRODUCTION: BASIC MECHANISMS

Ovulation Induction Results in Altered Growth and Metabolic Dysfunction in Mice Offspring
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MON-LB001

Nearly 15% of couples are affected by infertility and a large proportion of individuals will need to use ovulation induction (OI) or assisted reproductive technologies (ARTs) to conceive. Previous studies have shown that offspring conceived by ARTs are predisposed towards increased insulin resistance and glucose intolerance. However, the long-term effects of OI alone on offspring health have not been studied. This rodent study was designed to elucidate the effects of maternal supravoluntary on offspring growth and development.

C57Bl/6 females were either naturally mated (control= C) or super-ovulated (5 IU PMSG; 5IU hCG, OI group) and mated to C57Bl/6 males with one Agouti viable yellow (Avy) allelic mutation. The Avy allele contains an intracisternal A particle whose methylation levels determine expression of the agouti protein which alters coat color and can be used as a phenotypic readout for global methylation. Offspring (n= 108 control and n = 69 OI) were followed through 13 weeks of age to measure birth parameters, growth rate, fasting glucose, GTT, and body composition (EchoMRI). Parametric and non-parametric tests were used as indicated. Only results with p<0.05 are reported.

Results: Surprisingly, while litter size was not different (C = 7, OI = 6), superovulated mothers had fewer surviving pups (C=6.5 pups, OI=5 pups). No major differences in coat color frequencies were observed between the two groups, suggesting no changes in DNA methylation. All OI pups had decreased anogenital distance (males C = 2.1mm, OI = 1.7mm; females C = 1.74mm, OI = 1.48mm), while OI female had lower birthweights (C = 1.38g and OI = 1.23g). Starting at four weeks of age, OI male had lower weight compared to control males. As early as 3 weeks, significant differences in fasting glucose levels were noted (C = 162 mg/dL, OI = 149.5mg/dL). Additionally, superovulated males had lower lean mass at 8 weeks of age (tested by EchoMRI: C = 23.6g, OI = 19.5g) and higher insulin levels at 13 weeks (120 min post injection, C = 339 mg/dL, OI = 213 mg/dL). In summary, we found that the process of OI alone has profound effects on offspring development in a sexual dimorphic fashion. Additional studies will be performed up to 30 weeks of age. Funding: R01 HD092267-01to P

Genetics and Development (including Gene Regulation)

ENDOCRINE DISRUPTING CHEMICALS

Examination of Hepatic Gene Expression Following Developmental Exposure to Dieldrin in Trachemys Scripta and Discovery of a Novel Hepacivirus
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SAT-LB131

The Massachusetts Military Reservation (MMR) is a Superfund site where ground water has been contaminated by a mixture of pollutants. Exposure to these chemicals is a public health concern and reproductive impairments have been observed in a population of turtles (Chrysemys picta) endemic to this site. We hypothesize that developmental exposure to endocrine disrupting compounds originating from the MMR might lead to abnormalities seen in adult animals. Upon examination of egg yolk from turtles at the impacted site, we found the presence of dieldrin and p,p’-DDE. Turtles from a reference site were also found to have p,p’-DDE present in the yolk. In order to investigate these chemicals in the laboratory we used a closely related turtle (Trachemys scripta) and applied vehicle, dieldrin, or p,p’-DDE to the eggshells. Absorption of p,p’-DDE through the eggshell was limited. Although there were variations in absorbance, we were able to achieve levels of dieldrin in the yolk similar to what was seen in animals from the impacted site. Following in ovo exposure to dieldrin, we used RNAseq to examine hepatic gene expression in neonates and found that several transcripts were repressed at least 1.5-fold in the dieldrin-treated animals. QPCR was carried out to