and WBC count that mediate the body's inflammatory and immune responses (inflammatory mediators [IMs]). This relationship between inflammation and diabetes is complex, as statins have anti-inflammatory properties but paradoxically promote or exacerbate T2D. Recently it has been reported that beta-hydroxybutyrate levels characteristic of nutritional ketosis enhance cellular defenses against oxidative stress and block the assembly of the NLRP3 inflammasome. As part of an ongoing study of the effects of a well-formulated ketogenic diet (WFKD) delivered via a web-based continuous care intervention (CCI) on 262 patients with T2D[1] and 116 with prediabetes (PreD), we determined plasma levels of 16 IMs at baseline, 1 yr, and 2 yrs. These same IMs were concurrently monitored in 87 patients with T2D recruited as usual care controls (UC). At baseline, a statin was prescribed for 50% of the T2D/CCI patients, 27% of PreD/CCI patients, and 59% of the T2D/UC patients; at which time statin use was associated with reduced plasma CRP ($P=7 \times 10^{-5}$) compared to non-statin users in the T2D/CCI group only. There were no other significant baseline differences between statin users and non-users for any IMs (WBC, TNFa, IL-1b, IL-6, IL-8, IL-18, IFN-g, E- L-, and P-selectins, EGF, VEGF-A, MCP-1, ICAM-1 and VCAM-1). After 1 yr and 2 yrs of the CCI, mean weight losses in T2D were 12% and 10%, HbA1c reductions were 1.3% and 0.9%, and diabetes medication use was reduced by 51% and 53%, respectively. Linear mixed effects models were used to assess change in IMs over the 2 yrs, facilitating intent-to-treat analyses. Fourteen of the 16 IMs (excluding ICAM-1 and VCAM-1) were reduced compared to baseline in T2D/CCI ($P<0.001$), with none showing significant increases between yrs 1 and 2. A similar pattern albeit at lower magnitudes was seen in patients with PreD/CCI. Despite lower CRP values at baseline, T2D/CCI patients prescribed a statin experienced further reductions with the WFKD over the 2 years ($P=3 \times 10^{-5}$). In the T2D/UC group, no significant changes in any of the IMs were observed at 1 yr or 2 yrs. These observations suggest that a WFKD delivered via the CCI has broad-spectrum anti-inflammatory and immune modulatory effects in patients with T2D and PreD. Consistent with prior reports, statin use was associated with reduced CRP at baseline in the T2D/CCI group, but this effect was not significant in PreD/CCI and T2D/UC groups. CRP reductions were nonetheless significant in T2D/CCI statin users, suggesting added benefit of the WFKD. We conclude that improvements in IMs induced by a combination of nutritional ketosis and weight loss contribute to the beneficial effects of the CCI in the management of T2D.

1. Athinarayanan SJ, et al. Front Endocrinol. 2019. 5;10:348

# Diabetes Mellitus and Glucose Metabolism

## METABOLIC INTERACTIONS IN DIABETES

### *Mice With Skeletal Muscle-Specific DRP1 Deficiency Are Resistant to Obesity and Diabetes Induced by a High Fat Diet*

*Renato Daniel Jensen, BS, Joshua Peterson, BA, Benjamin Allington, BA, Alayna Dieter, BS, Linhai Cheng, BS, Jamie Soto, BS, Renata Pereira Alambert, PhD, Marcelo Correia, MD PhD, Evan Dale Abel, MD PhD.*
University of Iowa, Iowa City, IA, USA.

**SUN-LB118**

The skeletal muscle of type 2 diabetics exhibits mitochondrial dysfunction associated with increased mitochondrial fission. Dynamin-related protein 1 (DRP1) is responsible for mitochondrial division, whereas mitochondrial-endoplasmic reticulum contacts (MERCs) mark mitochondrial sites where fission occurs. Here, we have shown that skeletal muscle-specific DRP1 knock out (KO) mice are partly protected from high fat diet-induced obesity and diabetes, and exhibit increased insulin and glucose tolerance along with lower insulinemia. We also found that KO mice exhibit increased energy expenditure per unit of lean mass. Isolated DRP1-deficient skeletal muscle fibers from KO mice fed high fat diet have reduced respiratory capacity when exposed to ADP and palmitoylcarnitine, but not when exposed to ADP, pyruvate, and malate. Additionally, the skeletal muscle of KO mice fed normal chow exhibited altered expression of genes associated with MERCs and increased expression of genes linked to ER stress. We observed substantial increases in gene expression of FGF21, a downstream signal of the ER stress response, in KO mice. However, FGF21 plasma concentration in KO mice was not elevated. Additionally, changes in MERC gene expression could potentially alter calcium signaling between the mitochondria and endoplasmic reticulum, changing insulin sensitivity in KO mice. In conclusion, we have shown that skeletal muscle-specific DRP1 KO mice are resistant to high fat diet-induced obesity and diabetes, perhaps due to elevated energy expenditure and differential mitochondrial respiratory adaptations to different substrates. Although FGF21 does not appear to contribute to this effect, it is possible that other ER-stress signals might help explain the observed phenotype in KO mice.

# Healthcare Delivery and Education

## EXPANDING CLINICAL CONSIDERATIONS FOR PATIENT TESTING AND CARE

### *The "Normal" Hormonal Levels in Athletes: Should Reference Ranges Be Adapted for the Physically Active Population?*

*Flavio Cadegiani, MD, MSc, PhD[1], Tatiana P. C. Abrao, MD[1], Pedro Luiz H. da Silva, MD[1], Claudio E. Kater, Prof., MD, PhD[2].*
[1]Federal University of São Paulo, São Paulo, Brazil, [2]Federal University of Sao Paulo, Sao Paulo SP, Brazil.

**MON-LB305**

**Background:**Despite the growing number of physically active subjects, including elite and amateur athletes, little is known regarding metabolic and hormonal chronic adaptations to exercises. While the elucidation of the hormonal and metabolic physiological adaptations to physical activity is of emerging importance, the Endocrine and Metabolic Responses on Overtraining Syndrome (EROS) study have serendipitously unveiled the existence of multiple metabolic and endocrine physiological changes in male athletes, including chronic increase of testosterone with concurrent physiological increase of estradiol, enhanced GH and cortisol responses to stimulations,andincreased catecholamines, basal metabolic rate, fat oxidation, and hydration status. These findings were uncovered due to a novel methodological design in which athletes affected

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 adaptedranges 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 nMol/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 athleted should be interpreted with cautious, 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*

*Ian M. Gans, PhD Candidate[1], Ellen I. Hartig, B.S.[2], Andrea R. Tilden, PhD[3], Joel H. Graber, PhD[2], James Coffman, PhD[2].*

[1]University of Maine, Orono, ME, USA, [2]MDI Biological Laboratory, Bar Harbor, ME, USA, [3]Colby College, Waterville, ME, USA.

**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 glycolsis and peroxisomal beta-oxidation. Additional studies are required, but the regulation of glycolysis by Klf9 could contribute to this gene's known tumor-suppresive 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*

*Pandurangan Ramaraj, PhD.*

KCOM/A T Still University, Kirksville, MO, USA.

**SUN-LB27**

Previous clinical studies showed that menstruating females were better protected in melanoma than postmenopausal 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