SPX was effective in reducing food consumption with parallel drops in transcript expression of NPY, AgRP, NPY type 5 receptor (NPY5R) and ghrelin receptor (GHSR) in the hypothalamus, and these inhibitory effects could be blocked by GalR3 but not GalR2 antagonism. In agreement with the central actions of SPX, similar inhibition on food intake and hypothalamic expression of NPY, AgRP, NPY5R and GHSR could be observed with ICV injection of SPX. In the same study, parallel rises of transcript expression of leptin receptor (LepR) and melanocortin 4 receptor (MC4R) were also observed in the hypothalamus. These findings, taken together, suggest that the role of SPX as a satiety factor is well-conserved in the mouse. Probably, food consumption can induce SPX production in glandular stomach and contribute to the postprandial rise of SPX in circulation. Through GalR3 activation, this SPX signal can act within the hypothalamus to trigger feedback inhibition on food intake by differential modulation of the feeding regulators (NPY & AgRP) and their receptors (NPY5R, GHSR, LepR & MC4R) involved in the feeding circuitry of the brain.

Adipose Tissue, Appetite, and Obesity

NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

Spexin Friend or Foe? Novel Role of Spexin During Thermogenesis of White Adipose Tissue

Sabrina E. Gambaro, PhD, Guillermina Maria Zubiria, PhD, Alejandra P. Giordano, Doctoral Student, Ezequiel A. Harnichar, Doctoral Student, Andrea E. Portales, PhD, Andrés Giovambattista, PhD.
IMBICE (CONICET-CICPBA-UNLP), La Plata, Argentina.

Spexin (SPX) is a novel adipokine playing an emerging role in metabolic diseases due to its involvement in carbohydrate homeostasis, weight loss, appetite control, gastrointestinal movement, among others. Moreover, plasma levels are reduced in obese and type II diabetic patients. I*n vitro*, SPX favors lipolysis in adipocytes and hepatocytes and inhibits white adipogenesis. Therefore, the aim of this study was to evaluate the role of SPX in white adipose tissue (AT) thermogenesis. C57BL/6J male mice were treated or not with SPX for ten days (ip. 29 µg/kg/day; CTR and SPX). At day 3 mice were randomly divided: a group was kept at room temperature (RT) and the other at 4°C to stimulate thermogenesis (CTR-C and SPX-C). Caloric intake and body weight was daily recorded. At the end of the protocol plasma, Brown AT (BAT), abdominal AT (Epidydimal, EAT) and subcutaneous AT (Inguinal, IAT) depots were collected for several measurements. We found that caloric intake was increased when animals were exposed to cold ($P<0.001$). Body weight change revealed a differential effect of SPX depending on temperature (interaction SPX x Cold, $P<0.05$): SPX animals weighted less than CTR at RT, but upon cold stimulation there was no difference. No changes were observed for plasma glucose levels, however plasma triglycerides (Tg) levels decreased after cold exposure regardless SPX treatment (Cold $P<0.01$). Liver Tg content showed a SPX x Cold interaction effect ($P<0.0001$), where, upon cold stimulation, CTR-C animals increased their levels, but on the contrary SPX-C mice decreased it. EAT, IAT and BAT relative mass showed an interaction effect of variables (SPX x Cold $P<0.05$). When compared upon cold, SPX-C mice had less AT mass compared to CTR-C mice. IAT and EAT mRNA expression of UCP1 and Cox8b showed SPX x Cold interaction ($P<0.05$), with a tendency of reduction or no difference in SPX at RT, but with a significant decrease in SPX-C compared to CTR-C mice upon cold exposure. PGC1a expression was increased in EAT from cold exposed-mice and in IAT only in CTR-C mice. UCP1 protein levels showed different results depending on the AT depot. For IAT SPX x Cold interaction ($P<0.05$) was observed, where SPX inhibited UCP1 stimulation only upon cold exposure. On the contrary, for EAT UCP1 levels decreased in SPX-treated mice, regardless cold exposure (SPX $P<0.05$). In conclusion, SPX treatment *in vivo* reduced the thermogenic process in subcutaneous and abdominal AT, being more evident upon cold stimulation.
PICT2017-2038, PICT2017-2314.

Adipose Tissue, Appetite, and Obesity

NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

The Contribution of Arachidonic Acid Metabolites EETs to Inflammation in Obesity

Mona Mashayekhi, MD PhD[1], Celestine N. Wanjalla, MD, PhD[1], Christian M. Warren, BS[1], Joshua D. Simmons, BS[1], Samuel S. Bailin, MD[1], Curtis L. Gabriel, MD, PhD[1], Spyros A. Kalams, MD[1], Nancy J. Brown, MD[2], John R. Koethe, MD[1], James M. Luther, MD[1].
[1]Vanderbilt University Medical Center, Nashville, TN, USA, [2]Yale Medical Center, New Haven, CT, USA.

Background: Obesity is associated with increased prevalence of type 2 diabetes and cardiovascular disease (CVD). Adipose tissue (AT) contains a complex immune environment and is a central contributor to heightened systemic inflammation in obese persons. Increased chronic inflammation in obesity contributes to metabolic disease by increasing insulin resistance, and to CVD by causing an atherogenic dyslipidemia and increasing endothelial cell dysfunction and activation. Despite these links between inflammation and cardiometabolic disease in obesity, there are no current targeted therapies to prevent or reverse chronic inflammation in AT.
Epoxyeicosatrienoic acids (EETs) are lipid signaling molecules that act as potent vasodilators and promote sodium excretion in the kidney. Increasing EETs in rodents protects against hypertension and endothelial dysfunction. In humans, circulating EETs correlate with insulin sensitivity and are decreased in individuals with insulin resistance. EETs also decrease the inflammatory response to obesity in animal models, but the effect of EETs on inflammation in humans is currently unknown. EETs are hydrolyzed to less active forms by the enzyme soluble epoxide hydrolase (sEH), and we hypothesized that pharmacologic sEH inhibition with a specific inhibitor GSK2256294 (GSK) in obese patients would decrease AT inflammation.
Methods: Thirty-four obese prediabetic individuals were treated with placebo and GSK in a crossover design (NCT03486223). Participants had a seven-week washout in between drugs, and the order of drug was randomized and blinded. In a subgroup of patients, we collected

subcutaneous AT by liposuction and characterized T cell phenotypes by flow cytometry (N=7 paired samples).
Results: GSK decreased sEH activity in plasma (47.3% vs placebo; P=0.008) and in AT (58.8% vs placebo; P=0.002). GSK also decreased serum F2-isoprostanes (P=0.03), which are markers of oxidative damage and inflammation. In seven paired AT samples, T helper (Th) 1 cells producing the pro-inflammatory cytokine IFNγ were reduced by treatment with GSK as compared with placebo (% of total lymphocytes: Placebo 13.6% ± 6.9, GSK 11.0% ± 5.6, P=0.03 Wilcoxon Signed Rank). In this small sample, we did not detect significant differences in the percentage of other IFNγ-producing cells (natural killer: Placebo 19.0% ± 9.0, GSK 13.3% ± 4.9, P=0.18; CD8: Placebo 12.0 ± 11.0, GSK 6.1 ± 4.6, P=0.61). In addition, we did not detect any change in Th17, Th2, or regulatory T cells.
Conclusions: In a pilot study of seven individuals treated with placebo or an sEH inhibitor, we found that the sEH inhibitor decreased pro-inflammatory Th1 cells as compared with placebo in matched AT samples. Understanding the contribution of the EET/sEH pathway to inflammation in obesity could lead to new strategies to modulate AT and systemic inflammation and reduce the risk of CVD.

Adipose Tissue, Appetite, and Obesity
NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

The Effects of Diet Induced Obesity (DIO) on Skeletal Muscle Transcription in MuRF1 KO Mice

Andrea Gail Marshall, PhD[1], Jennifer E. Norman, Ph.D.[2], Michael S. Chementi, Ph.D.[1], John C. Rutledge, MD[2], Sue C. Bodine, Ph.D.[1].
[1]The University of Iowa, Iowa City, IA, USA, [2]The University of California, Davis, Davis, CA, USA.

Background: As obesity and Type II Diabetes rise globally, it is important to understand the similarities and differences in the response of metabolic tissues between males and females. We wanted to evaluate the impact of prolonged diet induced obesity (DIO) on the skeletal muscle transcriptome of our MuRF1 KO (KO) mice. **Methods:** RNA was isolated from the gastrocnemius muscle of male and female WT and KO mice that were fed either standard chow (Envigo 2918) or a 45% HFD (Research Diets D12451) for 22 weeks (n = 4). RNA was enriched for mRNA prior to library preparation. RNA sequencing was performed using 150 bp paired-end reads (~ 31.6 M reads per sample). Differentially expressed genes (DEGs) were identified using DESeq2 with an FDR set to 5%. **Results:** At baseline (chow diet), both male and female KO mice had DEGs compared to their WT counterparts (male, 1174; female, 105). Most DEGs were found to be unique by sex (male, 1151; female, 82), though 23 genes were found to be changed in common. After obesity was induced by 22 weeks of 45% HFD feeding, KO animals showed a greater transcriptional response than their WT counterparts. Males had 1821 DEGs (v. 179 in WT) while females had 4425 DEGs (v. 2090 in WT). In males, 78 genes were changed in common between WT and KO in response to DIO, with 76 of those genes changing in the same direction (Slc282a and Gm15427 did not). In females, 1445 genes were changed in common between WT and KO, with all but 2 genes (Pla2g7 and Zfp385b) changing in the same direction. In both male and female KO animals, oxidative phosphorylation and ribosomal pathways were most significant, though the direction of change in the DEGs was opposite. **Conclusion:** In skeletal muscle, sex highly influences the genes and pathways changed in response to DIO. Even among common pathways identified, the response between males and females differed. Loss of MuRF1 results in common and unique transcript changes in and between males and females under normal conditions and in DIO.

Adipose Tissue, Appetite, and Obesity
NOVEL MECHANISMS CONTROLLING ADIPOSE TISSUE PHYSIOLOGY AND ENERGY BALANCE

The Pesticide Chlorpyrifos Promotes Obesity by Inhibiting Diet-Induced Thermogenesis

Bo Wang, Ph.D.[1], Shuman Zhang, MS[1], Andrea Llanos, MS[1], Evangelia E. Tsakiridis, MS[1], Eric M. Desjardins, MS[1], Julian M. Yabut, PhD[1], Alexander E. Green, PhD[1], Emily A. Day, PhD[1], Brennan K. Smith, PhD[1], James S.V. Lally, PhD[1], Amogelang R. Raphenya, PhD[1], Krishna A. Srinivasan, PhD[1], Andrew G. McArthur, PhD[1], Shingo Kajimura, PhD[2], Jagdish Suresh Patel, PhD[3], Michael Gordon Wade, PhD[4], Katherine Mary Morrison, MD,FRCP[5], Alison Holloway, PhD[1], Gregory R. Steinberg, PhD[1].
[1]McMaster University, Hamilton, ON, Canada, [2]UCSF/Barnett Briggs Medical Lib, San Francisco, CA, USA, [3]University of Idaho, Moscow, ID, USA, [4]HEALTH CANADA HPFB, Nepean, ON, Canada, [5]McMaster University Medical Center, Dundas, ON, Canada.

Obesity is a major risk factor for type 2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease that arises from a caloric surplus of as little as 10–30 kcal per day. And while increased consumption of energy dense foods and reduced physical activity are commonly thought to be the major contributors to this caloric imbalance, diet-induced thermogenesis is a quantitatively important component of the energy balance equation. In adult humans, recent studies have indicated that diet-induced thermogenesis requires the activation of brown adipose tissue (BAT), however, the determinants regulating this process and why they may differ between individuals are not fully understood. We hypothesized that environmental toxicants commonly used as food additives or pesticides might reduce diet-induced thermogenesis through suppression of uncoupling protein 1, the defining protein of human BAT thermogenesis. Through a screening approach of pesticides/toxicants chosen from the Toxcast chem Library, we discovered that the organophosphate insecticide chlorpyrifos potently suppressed the expression of uncoupling protein 1 (UCP1) and mitochondrial respiration in brown adipocytes at concentrations as low as 1 pM. Chloropyrifos-induced suppression of brown adipocyte thermogenesis was also observed in mice fed a diet high in fat and housed at thermoneutrality where it promoted greater obesity, non-alcoholic fatty liver disease and insulin resistance. Reductions in thermogenesis by chlorpyrifos were associated with impaired activation of the β_3-adrenergic receptor and protein kinases critical for