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

Defining the Contribution of Adipocyte Subpopulations to Dermal White Adipose Tissue

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Introduction: Our lab has previously identified three distinct subtypes of developmentally and functionally white adipocytes and have shown that they each differentially contribute to adipose depots (Lee, KY et al. EMBO J. 2019). Dermal white adipose tissue (dWAT), a layer of adipocytes embedded in the skin below the dermis, has recently been shown to play a role in crucial physiologic processes including thermogenesis, the regulation of aging, scar formation, and wound healing. The purpose of this proposal is to investigate the contribution of three adipocyte subtypes to dWAT. **Objectives:** The primary objectives of this project are to determine the number of preadipocytes and adipocytes from each of three subtypes present in dWAT. **Methodology:** Lineage tracing analysis was performed by crossing transgenic mice harboring cre-recombinase under the control of promoter/enhancer elements of each of the three marker genes, Wilms tumor 1, transgelin, or myoxovirus 1 to dual- fluorescent reporter mice. These three mice lines mark Type 1–3 preadipocytes and adipocytes, respectively. dWAT was collected from X week old mice, and adipocyte identities were determined by confocal microscopy. Preadipocyte contribution of these subpopulations was determined by FACS analysis. **Results:** We found that Type 2 (~45%) and Type 3 (~25%), but not Type 1 preadipocytes significantly contributed to the dWAT preadipocyte cellular population. We also found a similar pattern for the adipocyte populations. Type 1, 2, and 3 adipocytes were found to comprise ~3%, 17%, and 7% of mature adipocytes, respectively. These studies demonstrate that Type 2 and Type 3 adipocytes contribute to the composition of dWAT. **Summary/Conclusion:** These studies demonstrate that Type 2 and Type 3 adipocytes and preadipocytes significantly contribute to the composition of dWAT. Since these adipocyte subpopulations have different functional properties, including metabolism and response to inflammatory cytokines, the contribution of these adipocyte subtypes may impact the crucial physiologic processes mediated by dWAT.

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Distinct Calcium Signaling for Wildtype, Loss-of-Function and Gain-of-Function Human MC4R Variants

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There is compelling evidence for human melanocortin-4-receptor (hMC4R) playing a critical function regulating energy balance; yet signal transduction pathways contributing to this are unclear. The hMC4R activates multiple signaling pathways, including induced increases in cAMP and mobilization of intracellular calcium ($[Ca^{2+}]$i). Recent evidence showed cAMP signaling was not a good predictor for hMC4R variant-associated obesity. We hypothesize that hMC4R mobilization of $[Ca^{2+}]$i plays an important role in regulating energy balance. To test this, we developed a robust high-throughput Fura-2 ratiometric fluorescent assay to quantitatively measure $[Ca^{2+}]$i *in vitro*. We compared basal and α-melanocyte stimulating hormone (α-MSH) activation of $[Ca^{2+}]$i for hMC4R-wildtype (WT) and hMC4R-variants stably expressed in HEK293 cells. The loss-of-function variants studied were two obesity-associated variants (R7H and R18L) known to exhibit cAMP signaling similar to WT, two obesity-associated variants (H76R and L250Q) known to exhibit cAMP-constitutive activity (CA) compared to WT, and one overweight-associated variant (H158R) known to exhibit cAMP-CA compared to WT. The gain-of-function variants (V103I and I251L) studied are known to exhibit cAMP signaling similar to WT. The data for basal $[Ca^{2+}]$i were pooled from three independent experiments performed with WT and all variants in each assay. Data (mean ± SEM) were analyzed using one-way ANOVA with Dunnett's multiple comparisons. The data (mean ± SEM) for α-MSH activation of hMC4R were pooled from three independent experiments and analyzed using non-parametric sum of squares F-test for maximum best-fit values and EC_{50}. The α-MSH activated assays were performed with each hMC4R variant alongside WT. WT hMC4R and non-CA loss-of-function variants exhibited similar basal and α-MSH activated $[Ca^{2+}]$i (WT: EC_{50} = 1.44 nM; R7H: EC_{50} = 1.40 nM; R18L: EC_{50} = 1.12 nM). The CA loss-of-function variants exhibited significantly ($p < 0.0001$) increased basal $[Ca^{2+}]$i compared with WT (WT = 97.6 ± 0.9 nM; H76R = 114.2 ± 1.7 nM; L250Q = 112.1 ± 2.6 nM; H158R = 110.7 ± 1.8 nM) and significantly lower EC_{50}'s compared with WT (H76R: EC_{50} = 0.07 nM; $p = 0.0019$; L250Q: EC_{50} = 0.09 nM; $p = 0.0066$; H158R: EC_{50} = 0.14 nM; $p = 0.0009$). The gain-of-function hMC4R variants exhibited significantly ($p < 0.0001$) decreased basal $[Ca^{2+}]$i compared with WT (WT = 97.6 ± 0.9 nM; V103I: = 86.4 ± 0.9 nM; I251L = 87.5 ± 1.0 nM) and significantly ($p = 0.0001$) increased α-MSH stimulated maximum $[Ca^{2+}]$i compared with WT (WT = 224.5 ± 13.6 nM; V103I = 288.2 ± 31.5 nM; I251L = 295.6 ± 20.0 nM). To summarize, we show three distinct patterns of hMC4R-associated calcium signaling; (1) WT and non-CA loss-of-function, (2) CA loss-of-function and (3) non-CA gain-of-function. Future studies are required to understand how hMC4R mobilization of $[Ca^{2+}]$i might contribute to the regulation of energy balance.

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Elevated Serum Uric Acid Is a Facilitating Mechanism for Insulin Resistance Mediated Accumulation of Visceral Adipose Tissue

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Background: Serum uric acid (SUA) is related to cardiometabolic conditions such as insulin resistance (IR) and visceral adipose tissue (VAT) accumulation, which have a thoroughly explored bidirectional relationship. Here, we aimed to clarify the nature of the role uric acid plays inside this relationship, alongside the underlying causality mechanism.
Methods: We conducted a population-based cross-sectional study comprising 8,504 subjects from a joint cohort composed from both NHANES 2003–2004 and 2011–2012 cycles and ENSANUT Medio Camino 2016. We performed mixed effects linear regression models using HOMA2-IR, adipoIR, and METS-VF as indicators of both peripheral and adipose tissue IR and VAT accumulation, indicating the subject's cohort of origin as a random effect. Furthermore, we performed multiple mediation analyses to assess a potential causal mechanism and ROC curves to establish cut-off points for identification of IR and visceral obesity using SUA. Finally, with an additional dataset comprised of 226 subjects with both euglycemic hyperinsulinemic clamp (EHC) and dual X-ray absorptiometry (DXA) measurements for IR and VAT accumulation, we performed a network of confirmatory mediation analyses including adiponectin measurements. **Results:** We found that SUA has a mediating role inside the bidirectional relationship between IR and visceral obesity, and it is part of an underlying causality mechanism which includes adiponectin. The proportion of the mechanism mediated by SUA is greater when stated that IR (in either peripheral or adipose tissue) leads to VAT accumulation (14.90%[13.20%-17.00%] and 15.54%[13.61%-18.00%]) instead of the opposite direction (4.88%[3.06%-7.00%] and 8.13%[5.91%-10.00%]). This result was strengthened by a mediation analysis network using the gold-standard measurements where we observed that the joint effect of SUA and adiponectin mediated 16.32% [8.84%-26.00%] for the effect of IR and VAT accumulation and 12.52% [3.23%-23.00%] in the opposite direction. Cut-off points for SUA to predict peripheral IR were 6.1 mg/dL and 4.8 mg/dL, for males and females respectively. For visceral obesity, cut-offs were 6.4 mg/dL and 4.8 mg/dL for males and females. SUA had a high negative predictive value for all assessments.
Conclusions: Elevated SUA acts as mediator inside the bidirectional relationship between IR and VAT accumulation. Its role appears to be larger when considering adipose tissue IR as the promoter for VAT accumulation.

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Environmental Enrichment Potentiates Glucose-Induced Anorexia in Mice

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The hypothalamus controls food intake and metabolism by integrating nutrient and hormonal signals from peripheral tissues. Both central and peripheral administration of glucose leads to a reduction in food intake in rodents. Similarly, administration of the adipocyte hormone leptin or the gastrointestinal hormone xenin reduces food intake. In contrast, impairments in hypothalamic signaling of these factors cause hyperphagia and obesity in rodents and humans. Environmental factors affect behavior including feeding behavior and energy metabolism in rodents and humans. Studies have found that environmental enrichment (EE), in which mice interact with complex sensory and motor stimulation, led to a significant reduction in adiposity and resistance to diet-induced obesity in mice. This effect is independent of energy expenditure and is associated with enhanced hypothalamic signaling, but the exact mechanism is unknown. We hypothesized that EE potentiates the feeding suppressing effects of anorectic signals. To address this hypothesis, 4-week-old male C57BL/6 mice were group housed (5/cage) under standard laboratory conditions or EE conditions with free access to regular rodent chow and feeding response to glucose, leptin and xenin was examined. EE cages were supplemented with a house, running wheels, igloos, wood logs, maze and nesting materials. Four weeks after initiating EE protocol, mice were fasted for 8 h and received an intraperitoneal injection of glucose (2 mg/g b.w.) or saline just before the onset of the dark phase. Treatment assignments were reversed for the second injection so that each animal received both treatments with a washout period of 1 week. Mice were given food immediately after the injection and food intake was measured for 4 h after the injection at 0.5–1 h intervals. The same design was repeated using leptin (2.5 μg/g b.w.) and xenin (15 or 50 μg/g b.w.). Glucose injection caused a significant reduction of food intake in both control and EE mice. However, anorexic effect of glucose was more significant in EE group compared to the control group (main effect of treatment: $P = 0.0016$ for control and $P < 0.0001$ for EE, two-way ANOVA). Significant reductions in food intake were observed between 0.5 and 2.5 h after glucose injection in EE mice, while no significant reduction was observed thereafter. Moreover, three-way ANOVA showed a significant interaction between housing condition and treatment ($P = 0.0086$). In contrast, although both leptin and xenin caused a significant reduction in food intake, there was no significant interaction between housing condition and treatment. These data suggest that environmental enrichment enhances the anorectic action of glucose without altering feeding response to leptin and xenin. It is speculated that enhanced hypothalamic glucose sensing may mediate beneficial effects of environmental enrichment on metabolism.

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Exposure to the Widely Used Pyrethroid Pesticide Deltamethrin, Does Not Exacerbate High Fat Diet Induced Obesity or Insulin Resistance in C57BL/6J Mice