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 Mara Leyendecker, BS¹, Rita Sharma, PhD², Quyen Luong, PhD¹, Noah Powell, BS², Brian Harper, BS², Kevin Y. Lee, PhD³. ¹Ohio University Heritage College of Osteopathic Medicine,

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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 (\sim 45%) and Type 3 (\sim 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, Lossof-Function and Gain-of-Function Human MC4R Variants

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There is compelling evidence for human melanocortin-4receptor (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 [Ca²⁺]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 [Ca²⁺]i *in vitro*. We compared basal and α -melanocyte stimulating hormone (α -MSH) activation of [Ca²⁺]i for hMC4R-wildtype (WT) and hMC4R-variants stably expressed in HEK293 cells. The loss-of-function variants studied were two obesityassociated 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 [Ca²⁺]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 \pm 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 $\text{EC}_{\scriptscriptstyle 50}$. The $\alpha\text{-MSH}$ 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 [Ca²⁺]i (WT: EC₅₀ = 1.44 nM; R7H: $EC_{50} = 1.40$ nM; R18L: $EC_{50} = 1.12$ nM). The CA lossof-function variants exhibited significantly (p < 0.0001) increased basal [Ca²⁺]i compared with WT (WT = 97.6 \pm 0.9 nM; H76R = 114.2 ± 1.7 nM; L250Q = 112.1 ± 2.6 nM; H158R = 110.7 \pm 1.8 nM) and significantly lower EC₅₀'s compared with WT (H76R: $EC_{50} = 0.07$ nM; p = 0.0019; L250Q: $EC_{50} = 0.09 \text{ nM}$; p = 0.0066; H158R: $EC_{50} = 0.14 \text{ nM}$; p = 0.0009). The gain-of-function hMC4R variants exhibited significantly (p < 0.0001) decreased basal [Ca²⁺]i compared with WT (WT = 97.6 ± 0.9 nM; V103I: = 86.4 ± 0.9 nM; $I251L = 87.5 \pm 1.0$ nM) and significantly (p = 0.0001) increased α -MSH stimulated maximum [Ca²⁺]i compared with WT (WT = 224.5 ± 13.6 nM; V103I = 288.2 ± 31.5 nM; $\rm I251L$ = 295.6 \pm 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 [Ca²⁺]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