

Supporting Information

The Ac-Trp-DPhe(p-I)-Arg-Trp-NH₂ 250-Fold Selective Melanocortin-4 Receptor (MC4R) Antagonist over the Melanocortin-3 Receptor (MC3R) Affects Energy Homeostasis in Male and Female Mice Differently

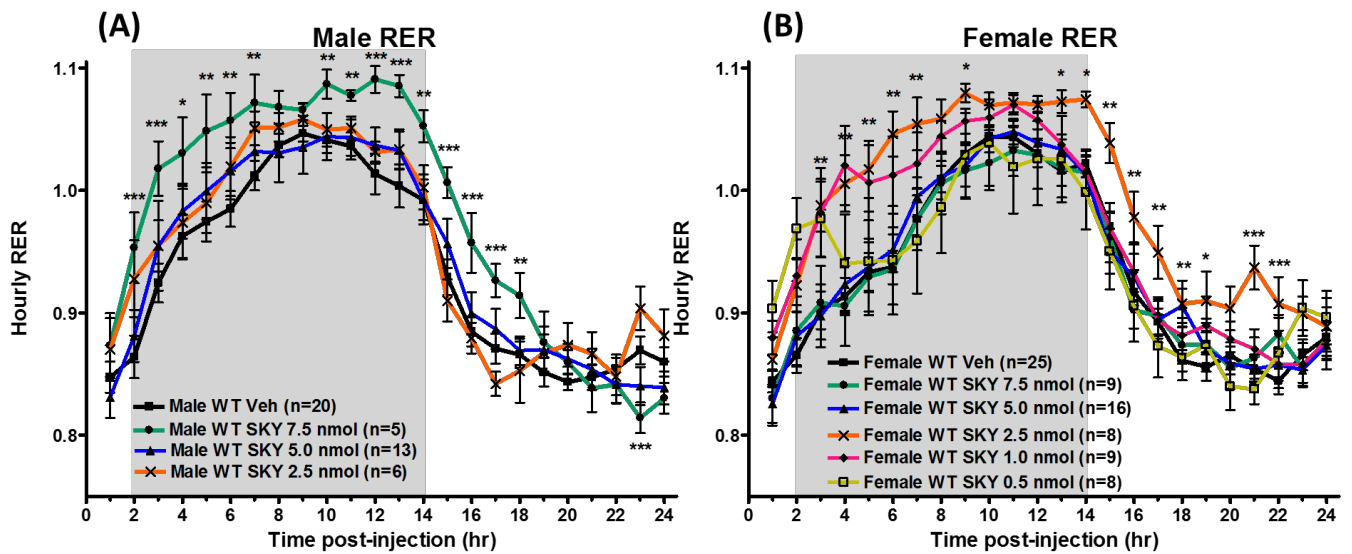
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Supporting Information. Exclusion Criteria (S1) TSE PhenoMaster Data Recording (S1-2) Male and Female Mice RER after SKY2-23-7 ICV Administration (All doses) (S2), Male and Female Mice Energy Expenditure after SKY2-23-7 ICV Administration (All doses)(S3), Male and Female Mice Activity after SKY2-23-7 ICV Administration (All doses)(S3), Latin-Square (crossover) feeding (S4)

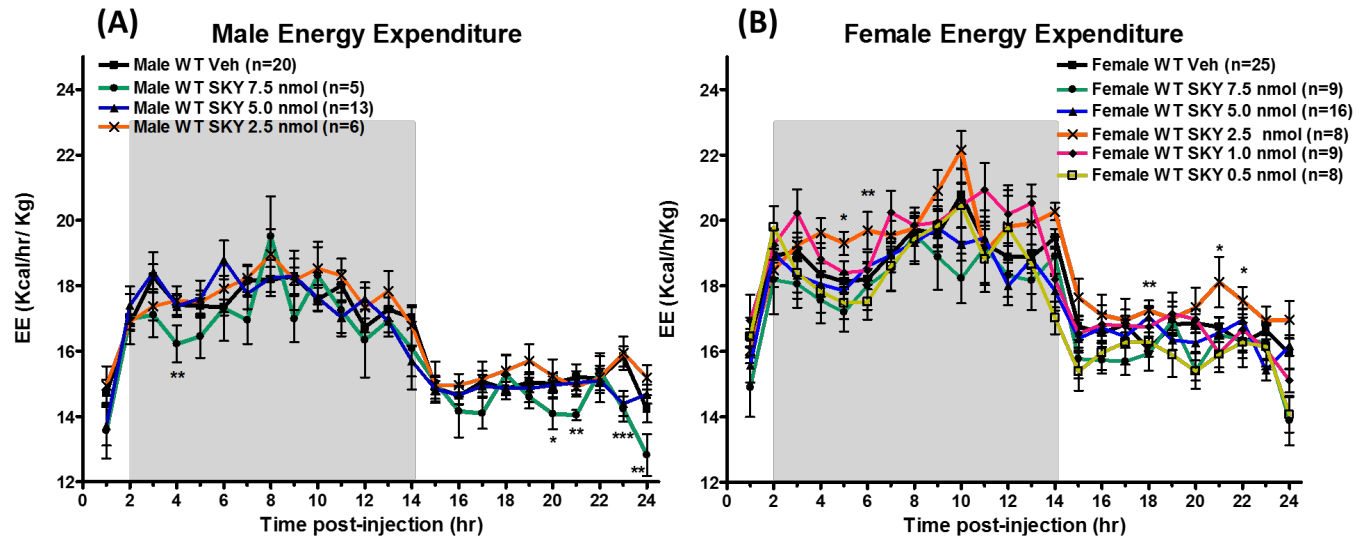
Exclusion Criteria- Mice which after administration recorded less than 1250 laser beam breaks in 8 hours were excluded from the study. This was a total of two mice, a male mouse after 7.5 nmol and 5 nmol administration (1235, and 500 beam breaks, respectively), and a female mouse after 5 nmol administration (596 beam breaks). Average beam breaks 8 hours post-administration was 6600±600 for males, and 6400±400 for females.

TSE PhenoMaster Data Recording: The TSE PhenoMaster system was configured to measure the food intake, water intake, oxygen uptake, carbon dioxide production, and locomotor activity in 15 minute bins. From the oxygen uptake and carbon dioxide production, the respiratory exchange ratio (RER) and energy expenditure were calculated. Cumulative food intake was reported in two hour increments. Activity, RER, and energy expenditure were reported in one

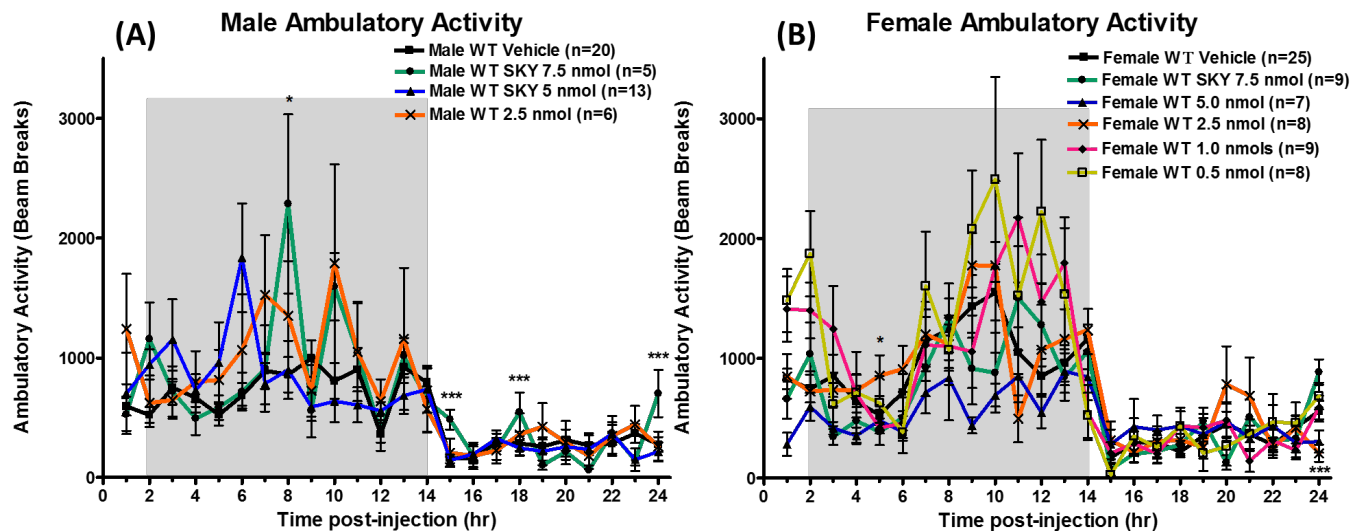
hour bins. The RER of four 15 minute recordings were averaged for each one hour bin. The four 15 minute recordings of energy expenditure (Kcal/hr) each hour was divided by the animals pre-treatment body weight (Kcal/hr/Kg) and were averaged for each one hour bin. Activity measurements were the ambulatory movement (X_A) recorded as the beam breaks of two different X axis beams. The activity recordings (X_A) over the four 15 minute recordings was summed for each one hour bin.



Supplemental Figure 1: Average hourly RER values of (A) males and (B) females after ICV administration of SKY2-23-7.



Supplemental Figure 2: Average hourly energy expenditure (EE) normalized to body weight (Kcal/hr/Kg) of (A) males and (B) female after ICV administration of SKY2-23-7.



Supplemental Figure 3: Average hourly activity of (A) males and (B) females after ICV administration of SKY2-23-7.

Supplemental Table 1: Experimental Design and Order of Compound Administration

| Time | Group 1 | Group 2 | Date |
|-------------|-----------------|-----------------|-------------|
| Week 1 | Surgery | Surgery | 10/6/14 |
| Week 2 | hPYY | Saline | 10/14/14 |
| Week 3 | Saline | hPYY | 10/21/14 |
| Week 4 | TSE Acclimation | TSE Acclimation | 10/28/14 |
| Week 5 | Vehicle | SKY 5 nmol | 11/6/14 |
| Week 6 | SKY 5 nmol | Vehicle | 11/12/14 |
| Week 7 | Vehicle | SKY 2.5 nmol | 11/18/14 |
| Week 8 | SKY 2.5 nmol | Vehicle | 11/24/14 |
| Week 9 | Vehicle | SKY 7.5 nmol | 12/02/14 |
| Week 10 | SKY 7.5 nmol | Vehicle | 12/10/14 |
| Week 11 | SKY 5 nmol | SKY 5 nmol | 12/18/14 |
| Week 12 | SKY 1 nmol | SKY 1 nmol | 12/24/14 |
| Week 13 | SKY 0.5 nmol | SKY 0.5 nmol | 1/7/14 |