**NEGATIVE REGULATION OF FOOD INTAKE BY JNK1**

**Introduction**

c-Jun-N-terminal kinase (JNK) is a ubiquitously expressed serine kinase that is activated by proinflammatory cytokines and stressors as well as metabolic stimuli: glucocorticoids (cort), free fatty acids and insulin. Many of these signals also act on hypothalamic neurons to affect feeding. The isoform JNK1 is a negative regulator of insulin signaling by serine phosphorylation of IRS proteins. JNK1 null mice are lean, insulin hypersensitive and resistant to diet-induced obesity.

**Elevated Agrp expression**

The hypothalamus contains two populations of neurons that dynamically regulate feeding behavior and energy expenditure: anorexigenic Pomc neurons promote negative energy balance and orexigenic Agrp neurons promote positive energy balance. Because JNK1 -/- mice are lean it is likely they have defects in these neuropeptides.

**Elevated GR activity**

Glucocorticoid (cort) is a well known stimulator of food intake and Agrp expression. In vitro studies have shown that JNK1 directly phosphorylates its receptor, glucocorticoid receptor (GR), inhibiting its function by affecting protein stability, nuclear translocation, sumoylation, and transcriptional activity. GR is a nuclear hormone receptor whose expression is enriched in the mediobasal hypothalamus. Fasting-induced stress greatly increases circulating cort levels and thus GR signaling. It is possible that the elevated Agrp expression is due to increased GR activity.

**Central DEX induces hyperphagia**

Because JNK1 is ubiquitously expressed it is necessary to determine whether these effects are secondary to peripheral changes or direct actions on the hypothalamus. In order to test this, JNK1-/- mice were fed or fasted for 36h, then perfused and stained with GR. Nuclear localization indicates activation. Plasma cort levels were measured on fed or 36h fasted mice after rapid decapitation by decorticosterone EIA. Cort n = 5-6, GR n = 3 (2-3 sections each). * P < 0.05, ** P < 0.01 by Student’s t-test.

**Excessive refeeding**

The fasting-refeeding paradigm is a functional test of the ability to recover from a period of starvation. Because fasting causes high stress and JNK1 -/- mice have greater GR activation in Agrp neurons, they should show exaggerated hyperphagia during this period.

**JNK inhibition stimulates Agrp**

Since centrally administered DEX was able to stimulate a hyperphagic response in JNK1 -/- mice so in order to evaluate Agrp expression, hypothalamic explants were used. This eliminates the possibility of peripheral contribution and by halving each explant, allows for pairwise comparisons.

**Agrp ablation prevents hyperphagia**

If JNK inhibition increases Agrp expression, its effects should be abolished in the absence of Agrp. Agrp-Tfam mutants exhibit progressive ablation of Agrp neurons in such a way that no major body weight or food intake phenotype is detectable. However, they should not respond to DEX’s hyperphagic effects when treated with JNK inhibitor.

**Conclusion**

JNK1 acts to negatively regulate short-term food intake through GR activation in Agrp neurons. Despite their long-term body weight phenotypes, JNK1 -/- mice are hypersensitive to glucocorticoid’s central hyperphagic effects because of increased GR activation leading to increased Agrp expression. These results can be recapitulated both in vitro and in vivo using a JNK inhibitor but only when Agrp neurons are present.