Insensitivity to the feeding response of exogenous ghrelin develops during pregnancy

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Introduction

The maternal body undergoes many metabolic adaptations, to support the energy demands of pregnancy and lactation. Some of these adaptations include resistance to peripheral satiety signals, CCK, insulin and leptin to increase food intake (Figure 1). Ghrelin, is an orexigenic hormone, which activates agouti-related peptide (AgRP) neurons in the arcuate nucleus to promote rapid food intake. Here, we investigate the contribution of ghrelin in elevated maternal food intake.

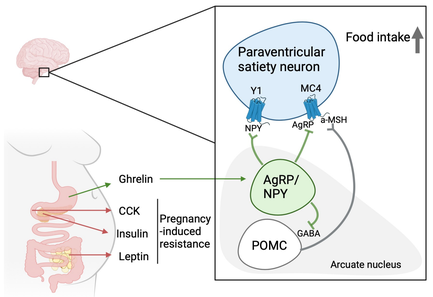

Figure 1. Food intake is increased during pregnancy partially due to the central resistance of satiety signals, CCK, insulin and leptin. Ghrelin stimulates food intake by activation of AgRP neurons.

Materials and Methods

Ghrelin induced food intake: Following 5 hours of food deprivation in the light phase, Female C57BL/6 mice were injected (i.p.) with either ghrelin (0.3mg/kg) or vehicle (saline) at four physiological timepoints: prior to pregnancy (virgin), day 8 and 15 of pregnancy and day 10 of lactation then 2h food intake was measured.

Immunohistochemistry: Female AgRP-cre x td Tomato mice were injected with either ghrelin (0.3mg/kg i.p.) or saline at three physiological timepoints: prior to pregnancy (virgin), day 15 of pregnancy and day 10 of lactation and food was removed. After 2 hours mice were perfused with 4% paraformaldehyde and brains were processed for c-fos immunofluorescent labelling.

Fiber photometry: AgRP-cre female mice underwent stereotaxic surgery to deliver an adeno-associated virus (AAV9) containing the cre-dependent GCaMP6s. A unilateral injection of the AAV was administered into the ARC followed by a fiberoptic cannula.

AgRP neuron activity testing paradigm: AgRP neuron activity was recorded in the early light phase of metestrus (virgin), pregnancy day 8, pregnancy day 15 and lactational day 10. A baseline consisting of 10 minutes of AgRP neuron activity was recorded followed by 10 minutes of the response to ghrelin (0.3mg/kg i.p.) and lastly chow. For analysis, Z- score normalisation was used. This calculates the standard deviations a data point is away from the defined baseline mean. Z = (Δ F/F value – mean of the baseline period)/ standard deviation of the baseline period

Results

Ghrelin does not induce acute food intake in pregnancy

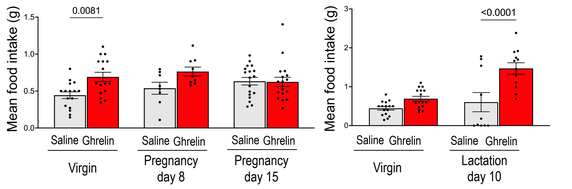

Figure 2. A Food intake over 2 hours after ghrelin injection in virgin, day 8 and pregnant and day 15 pregnant mice (two-way ANOVA, Interaction $p = 0.0482$, Šídák's multiple comparisons $p = 0.0081$). **B** Food intake over 2 hours in virgin and lactating mice after ghrelin injection (two-way ANOVA interaction effect of treatment x reproductive state $p = 0.0150$, Šídák's multiple comparisons $p < 0.0001$).

Ghrelin activates AgRP neurons in virgin, pregnant and lactating mice

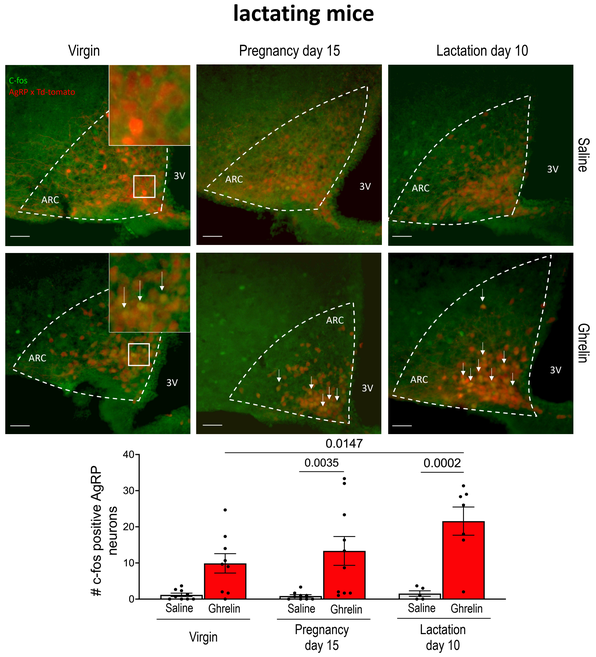

Figure 3. A Representative images of endogenous td-Tomato indicative of AgRP neurons and c-fos immunostaining in the ARC after vehicle or ghrelin injection. White arrows indicate co-localisation. Scale bar 200 µm, 3V third ventricle. **B** C-fos expression co-localised with AgRP neurons in response to vehicle or ghrelin injection in virgin, day 15 pregnant and day 10 lactating mice (two-way ANOVA Effect of treatment p <0.0001 Šídák's multiple comparisons ghrelin: virgin vs lactation day 10 $p = 0.0147$. Pregnancy day 15: saline vs ghrelin $p = 0.0035$, Lactation day 10 saline vs ghrelin $p = 0.0002$).

Ghrelin rapidly increases AgRP neuron activity in virgin and pregnant mice

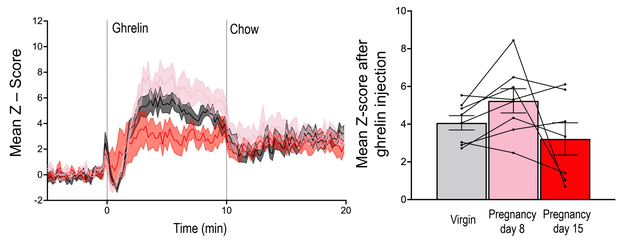

Figure 4. A,B AgRP neuron activity in response to ghrelin in virgin, day 8 pregnant and day 15 pregnant mice. **B** Average Z-score of AgRP neuron activity in the 10-minute period following ghrelin injection (RM one-way ANOVA $p = 0.1248$). Graphs displayed as mean ± SEM, N = 8.

Ghrelin rapidly increases AgRP neuron activity in lactating mice

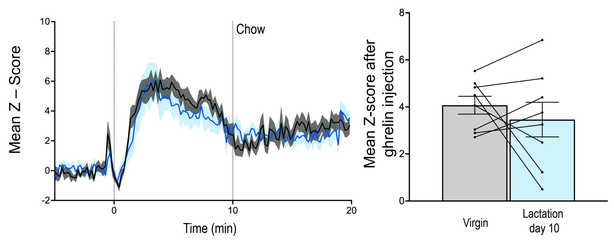

Figure 6. A,B AgRP neuron activity in response to ghrelin in virgin and day 10 lactating mice. **B** Average Z-score of AgRP neuron activity, in the 10-minute period following ghrelin injection (Paired t-test $p = 0.4637$). Graphs displayed as mean ± SEM, N = 8.

Chow-induced decreases of AgRP neuron activity is blunted on day 15 of pregnancy

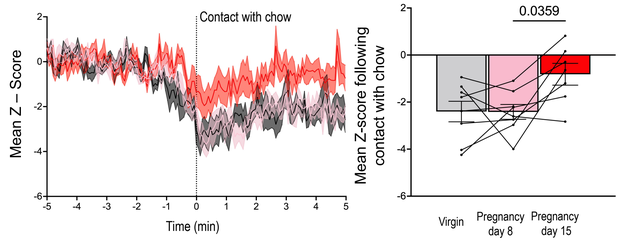

Figure 5. A,B Average AgRP neuron activity in response to chow in virgin, day 8 pregnant and day 15 pregnant mice. **B** Mean AgRP neuron activity 5 minutes after contact of chow (RM one-way ANOVA $p = 0.0208$). Graphs displayed as mean ± SEM, N = 8.

Chow does not decrease AgRP neuron activity in lactating mice

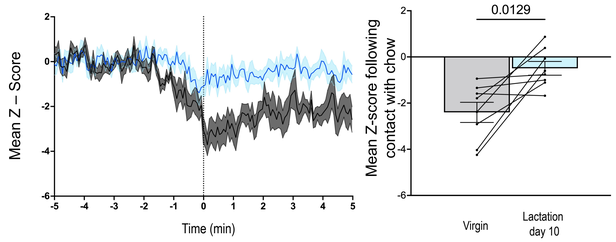

Figure 7. A,B Average AgRP neuron activity in response to chow in virgin and day 10 lactating mice. **B** Average Z-score of AgRP neuron activity 5 minutes after contact of chow (Paired t-test $p = 0.0129$). Graphs displayed as mean ± SEM, N = 8.

Conclusions

- During pregnancy, exogenous ghrelin does not stimulate increased food intake indicating a state of ghrelin insensitivity develops.
- This insensitivity is not mediated by the AgRP neuron population in the ARC as they are still responsive to ghrelin.
- Ghrelin insensitivity is specific to pregnancy as the food intake response to ghrelin is restored in lactation.
- During lactation AgRP neurons show a lack of response to consumption of chow suggesting that adaptations occur to increase food intake.

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