

signalling pathways activated by INSL5 at RXFP4 is essential for understanding the biological roles of this novel gut hormone.

## Reference

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### Insights into the trajectory of neuronal projections to brown adipose tissue derived from the use of novel “brainbow” neurotropic viruses

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The medullary raphe nuclei are regarded as the final common neural relay connecting higher brain centres to the spinal cord and sympathetic outflow to brown adipose tissue (BAT), an observation which is based on functional (electrophysiological) data. In order to define the trajectory of populations of neurons in the hypothalamus, synaptically linked to the medullary raphe nuclei and ultimately BAT, a novel approach was utilised which involves the injection of a modified form of pseudorabies virus (PRV) into the BAT. This changes the colour of its fluorescent reporter when it comes into contact with Cre recombinase. We hypothesise that contrary to the dogma stated at the outset there will be two populations of neurons projecting to BAT, one which passes through the midline raphe and the other that involves alternate premotor pathways in the brainstem.

An (AAV)-Cre recombinase construct was injected stereotaxically into the raphe nuclei of male Sprague Dawley rats weighing between 230 and 250g. This injection was followed 2 weeks later by injection of PRV Brainbow virus (PRV-263) into the interscapular BAT. After 4 days survival, rats were killed and their brains prepared for histological analyses.

PRV-263 which is replication competent was transported retrogradely from the BAT through chains of synaptically-connected neurons in the spinal cord, brainstem and hypothalamus including the midline raphe. After transport through neurons expressing Cre recombinase in the raphe there was recombination of the viral genome



at either paired lox2272 or loxP sites, resulting in the loss of the red reporter and expression of either cyan (mCerulean) or yellow (eYFP). Importantly there were distinct groups of neurons in the rostroventrolateral medulla, lateral hypothalamus and paraventricular nucleus that retained their red fluorescent reporter consistent with a trajectory other than through the raphe nuclei.

These data define the nature of descending neural projections to BAT.

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### Oleylethanolamine and endocannabinoid responses to intraduodenal lipid infusion in humans: Relationships with BMI and energy intake

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**Introduction/aims:** Fat stimulates intestinal secretion of oleylethanolamide (OEA) to reduce food intake, while fasting induces intestinal endocannabinoids with an orexigenic effect. Animal studies suggest that high-fat diet-induced obesity impairs intestinal control of endocannabinoid and OEA production, contributing to reduced satiety and weight gain. The aims of this study were to: (i) evaluate effects of intraduodenal (ID) lipid infusion on plasma levels of anandamide (AEA), 2-arachidonylethanolamide (2-AG) and OEA in humans, and to examine relationships with BMI and *ad libitum* energy and fat intakes, and (ii) to evaluate effects of ID lipid on duodenal concentrations of 2-AG, AEA and OEA.

**Methods:** 19 lean, 16 overweight and 17 obese participants underwent ID Intralipid<sup>®</sup> infusion (2 kcal/min) for 120 min during which blood samples were collected every 30 min. *Ad libitum* energy intake was assessed at a subsequent buffet meal. Endoscopic duodenal biopsies were collected from 4 lean participants, at baseline, and following 30 min ID Intralipid<sup>®</sup> infusion (2 kcal/min). Plasma and duodenal 2-AG, AEA and OEA concentrations were assessed by HPLC/tandem mass spectrometry



**Results:** There were positive relationships between fasting plasma 2-AG ( $r=0.4$ ,  $P=0.009$ ) and AEA ( $r=0.3$ ,  $P=0.018$ ), but not OEA, with BMI. ID lipid had no effect on plasma concentrations of 2-AG or AEA, but there was a group  $\times$  time interaction for OEA ( $P=0.026$ ). OEA increased in response to lipid in the obese, but not the lean or overweight groups (post-hoc  $P=NS$ ). There was a relationship between plasma OEA at  $t=120$  min with energy ( $r=0.35$ ,  $P=0.022$ ), but not fat, intake at the buffet meal. 30-min of ID fat infusion significantly increased duodenal OEA ( $P=0.046$ ), but had no effect on 2-AG or AEA concentrations.

**Conclusions:** Fasting endocannabinoid tone, and lipid-induced OEA secretion appear altered in human obesity. Duodenal mobilisation of OEA may play an important role in the regulation of food intake.

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### Weight loss and tissue remodelling following 8-week calorie restriction or intermittent fasting in females who are overweight and obese



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Tissue remodelling and changes in macrophage polarisation occur in adipose tissue (AT) and skeletal muscle (SM) in response to nutrient availability in animals [1–4]. This study aimed to compare the effects of daily calorie restriction (CR) versus intermittent fasting (IF) on markers of AT and SM remodelling in women.

Women ( $N=75$ ,  $50.3 \pm 1.0$  y, BMI  $32.4 \pm 0.5$  kg/m<sup>2</sup>) were randomised to 1 of 3 groups for 8 weeks, and provided with foods at 70% (IF70 and CR70), or 100% (IF100) of energy requirements. CR70 participants consumed food daily, whereas IF participants ate breakfast, prior to initiating a 24-h fast, for 3 non-consecutive days/week. Fasting bloods, subcutaneous abdominal AT and quadriceps muscle biopsies were obtained at baseline, and 8 weeks, after a 12-h overnight fast (all groups), and 24-h fast (IF groups). Markers of macrophages

and extracellular matrix (ECM) were examined by qPCR.

We observed significant weight loss after 8 weeks, with greater weight loss in IF70 vs. CR70 and IF100 ( $P < 0.05$ ). Insulin sensitivity, assessed by HOMA-IR, was improved in IF70 and CR70 following a 12-h fast and in all groups following a 24-h fast, with greatest improvement in IF70 ( $P < 0.05$ ). Reductions in NEFAs were greater in IF70 vs. CR70 after a 12-h fast, whereas NEFAs increased after a 24-h fast in IF groups ( $P < 0.05$ ). In AT, CD40 expression (M1-macrophage marker) was increased following a 24-h fast in IF70 and MMP2 (involved in breakdown of ECM), was increased in CR70 and IF70 ( $P < 0.05$ ). In SM, CD163 (M2-macrophage) expression was increased after 12- and 24-h fasts in fasting groups ( $P < 0.05$ ).

Weight loss stimulated markers of ECM remodelling in AT. IF increased pro-inflammatory M1 macrophages in AT and anti-inflammatory M2 macrophages in muscle. We speculate this increase in macrophages may be an appropriate response to buffer increased lipolysis in response to severe energy deprivation.

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### The influence of meals containing differing fatty acid compositions on appetite parameters in overweight and obese individuals



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**Introduction:** It has been proposed that differing dietary fats are capable of modulating appetite signalling, possibly altering the amount of food consumed at subsequent meals, and in the long term, body weight.

**Methods:** Eight normoglycaemic overweight or obese individuals (BMI  $32 \pm 1.2$  kg/m<sup>2</sup>) randomly completed three single-blinded, fasting breakfast