***Project title***

Examining the target of GDF15 in the brain: GFRAL neurons driving physiology and behaviour

***Project summary***

*I request that this report please be considered in light of the constraints of the COVID19 pandemic, specifically the two Melbourne lockdowns including the closing of schools necessitating home learning for my primary school-aged child for most of term 2 and all of term 3, and the general reduced capacity for research in 2020.*

Central regulation of metabolic disease has almost exclusively been explored in the context of obesity. This is understandable given prevalence rates, however diseases of undernutrition also represent a significant health burden. This is especially so with regard to cancer cachexia-anorexia syndrome (cachexia). Cachexia is a progressive loss of body weight, accompanied by loss of appetite, which affects the majority of cancer patients. Cachexia is directly responsible for 20-30% of all cancer deaths [4] and, as well as affecting patient mortality, cancer cachexia increases surgical risk, reduces effectiveness of chemo-/radiotherapy, predisposes patients to mood disorders, and reduces quality of life. There is currently no effective treatment. *One key issue for this is a lack of understanding of how the brain fails to maintain energy homeostasis during cachexia.*

One novel candidate for *direct central regulation* of cachexia is growth and differentiation factor 15 (GDF15). A transforming growth factor beta (TGFb) superfamily cytokine, it is secreted from the cells of most cancers and causes anorexia/cachexia in mice [5]. Its receptor, Glial cell line-derived neurotrophic factor (GDNF)-family receptor α-like (GFRAL), is expressed solely in the brainstem [6]. This receptor is recently discovered, and importantly localises the effects of GDF15 to the brain. GDF15 KO mice are obese [7], and GFRAL KO mice show increased weight gain on a high fat diet [6], firmly suggesting this pathway is important for physiological regulation of body weight as well as pathological dysregulation. Moreover, GFRAL activation by GDF15 results in Fos activation [6] in areas shown to be important in mood related behaviour in cachectic mice [8]. This suggests that the neural circuits activated by GDF15-GFRAL signalling regulate both mood and metabolic processing*.*

Pathologically, GDF15 is increased in diseases of tissue stress, such as congestive heart failure, kidney disease [10], and cancer. These diseases involve mood and motivational impairments [11] and the conventional wisdom has been that these associations secondary consequences of the emotional reaction to the disease [12], however the possibility that there is a mechanistic connection between nausea/feeding pathology and mood/motivation disturbances has not been examined.GDF15 has been identified as a key driver of decreased food intake in cancer cachexia [13, 14]. Cachexia is associated with mood disturbances, and symptoms (muscle wasting and malnutrition) significantly correlate with increased depression and anxiety scores [16], even in newly diagnosed patients [17]. Changes to mood and motivation are characteristic of diseases associated with increased GDF15 [11].

This project aimed to address directly the role of direct activation of the GFRAL-containing neurons using a novel mouse line expressing cre recombinase only in GFRAL neurons. Using this cre line, I aimed to use Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to inhibit and activate this neuronal population and then assess changes in mouse behaviour and metabolic physiology. Unfortunately, validation of the novel mouse line to be used in this project uncovered issues with cre expression in the GFRAL neurons. After trying 3 separate methods to validate cre expression in these mice, we concluded they were not expressing cre in the GFRAL neurons. Further investigation uncovered incorrect phenocopy of the transgene in these animals transferred to us from the supplier, and the line was terminated. To fulfil the aims of this project, I instead used a method already available to me – using an adeno-associated virus to drive overexpression of GDF15 specifically in the hindbrain, with a GFP-expressing virus serving as the control. This virus was the gift from Dr. Kelly Walton, and I thank her for sharing it.

***Aim 1: Effects on mood-related tests and motivated behaviour***

In this study, we assessed the capacity of overexpression of GDF15 to promote changes in a range of behavioural paradigms. Anxiety-like behaviour was assess using standard rodent tests – the elevated plus maze, the open field and the light-dark box. In all tests, GDF15 overexpressing mice showed no changes in locomotor activity, speed, distance travelled or willingness to explore open areas. This indicates no changes to anxiety-like behaviour in mice with tonic activation of GFRAL by GDF15. Overexpressors showed a significantly increased sensitivity to taste aversion induced by LiCl, indicating a heighted response to noxious stimuli.

We assessed how willing mice were to work for a sugar reward using an in-cage operant conditioning device (Feeding Experimentation Device; FED3). Contrary to expectations, GDF15 overexpressors showed an increased willingness to work for a sugar reward, indicating they are more responsive to the motivational effects of sugar. This increase may be secondary to body weight loss in the GDF15 overexpressors, a known factor in motivational responding. We are currently repeating this experiment to investigate how GDF15 overexpression influences motivated responding in calorie restricted mice, to rule out hunger or weight loss as the primary drivers.

***Aim 2: Effects on food intake and metabolic rate***

In this study, we assessed how overexpression of GDF15 alters appetite and thermogenic response in response to metabolic challenge. After injection of the GDF15-encoding transgene, mice were implanted with radio-telemeters into the interscapular brown adipose tissue (BAT). These telemeters allow for minute to minute recording of BAT temperature as well as locomotor activity. At baseline, mice overexpressing GDF15 had similar BAT temperature to GFP-injected control mice. However, when temperature was recorded during dark-phase fasting GDF15 overexpressors showed a delayed drop BAT temperature, indicating a reduced ability to adapt to the fasted state by reducing energy lost through heat production. Interestingly, this was not seen following an injection of the hormone ghrelin, a key signal of hunger to the brain. Recent publications have demonstrated that GFRAL is located in neurons also containing cholecystokinin, a key anorectic peptide, so this population may be more responsive to other signals if negative energy balance.

***Presentations relating to this award:***

63rd Endocrine Society of Australia Annual Scientific Meeting (Virtual)

**Overexpression of GDF15 in the brainstem drives increased brown adipose tissue thermogenesis, and increases sensitivity to taste aversion while decreasing preference for sweet taste.**

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