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Coordinated activation of central amygdala–arcuate nucleus NPY circuits are required for the development of stress-induced obesity

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Neuropeptide Y (NPY) is one of the most powerful orexigenic peptides, exerting critical feeding related functions in the hypothalamus. However, NPY is also present in extra-hypothalamic nuclei where it is modulated in response to metabolic and other physiological perturbations, but far less is known about these NPY populations and their influence on energy homeostasis. Under conditions of high-fat-diet (HFD) feeding in combination with chronic stress (HFDS), the expression of NPY is robustly upregulated in the central amygdala (CeM) as well as in the arcuate nucleus (Arc), accompanied by an increase in HFD consumption resulting in a significant increase in body fat mass, and a decrease in energy expenditure (EE). To delineate the functional role of CeM-NPY under HFDS condition, we overexpressed NPY specifically in NPY neurons of the CeM in mice. This resulted in increase in food intake and diet-induced EE, subsequently leading to an increase in body fat mass. Importantly, specific ablation of NPY in the neurons of the CeM significantly reduced the obesity-associated phenotype, confirming the importance of NPY in the CeM for the regulation of stress-induced obesity (SIO). Not surprisingly, HFDS-treated mice are also less insulin sensitive with strongly reduced glucose tolerance. Under unstressed situation, insulin reduces the level of NPY in the CeM, which leads to the reduction in food intake in mice, exhibiting an anorectic action. However, our results show that this anorectic action of insulin on the CeM-NPY neurons is impaired under HFDS condition, exaggerating the development of diet-induced obesity. In summary, our data provide important new insights to the contribution of CeM-derived NPY in stress-induced feeding and also identifies the underlying mechanism for the development of obesity under combined high caloric food intake and stressful conditions.

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Development of biomarkers for protein malabsorption and *in vivo* inhibition of B^0AT1(SLC6A19)

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Recent studies have established that dietary protein restriction has beneficial impacts on the metabolic health and it promotes improved glucose homeostasis by increasing the induction of FGF21 from liver. B^0AT1 (SLC6A19) is the major neutral amino acid transporter in the intestine that carries out the bulk of amino acid absorption from the diet. It also reabsorbs neutral amino acids in the proximal tubule of the kidney. Mice lacking B^0AT1 show signs of protein restriction such as elevated levels of FGF21 and reduced mTORC1 activity. Moreover they have improved glucose homeostasis and are protected from diet induced obesity. Pharmacological blockage of this transporter is expected to replicate this phenotype and it could be employed to treat metabolic diseases such as type 2 diabetes. To evaluate the efficacy of inhibitors of B^0AT1, we aimed to develop biomarkers that can reliably detect successful inhibition of B^0AT1 in mice *in vivo*. B^0AT1 wildtype and knockout mice served as surrogates for control and fully inhibited B^0AT1 activity. Lack of B^0AT1 was confirmed by delayed plasma appearance rates of [^{14}C]-labelled neutral amino acids compared to wildtype mice after intragastric administration of a mixture of amino acids. A GC-MS based non targeted metabolomics approach was used to discriminate metabolites profiles in plasma, urine and faecal samples. Lack of B^0AT1 was readily detected by significantly increased abundance of neutral amino acids in urine and faecal samples. Metabolites of bacterial protein fermentation such as cresol, 3-indole-propionic acid, 3 hydroxyphenylacetate were more abundant in B^0AT1 knock out mice, indicating protein malabsorption of dietary amino acids. ROC curve analysis was used to validate the use of these metabolites as biomarkers. These findings provide putative metabolite biomarkers that can be used to detect protein malabsorption and the inhibition of this transporter in intestine and kidney.

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The role of Nox5 in diabetic complications

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Adiposity and spatial memory impairments are determined by the pattern of access to high-fat, high-sugar 'cafeteria' diet in rats

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Rising rates of disordered eating behaviours in Australia (e.g., bingeing and excessive restriction of intake) suggest that repeated 'cycling' between healthy and unhealthy eating is increasingly common. The effects of such cycling on obesity and cognitive outcomes are unknown. Here we used rats to assess the effects of cycling between chow and a cafeteria (CAF) diet rich in saturated fat and refined carbohydrates on the development of obesity and spatial

recognition memory. In Experiment 1, rats fed a healthy chow diet (control group) were compared with groups fed CAF for: 3 consecutive days per week followed by 4 days of chow (3:4 group); 5 consecutive days per week followed by 2 days of chow (5:2 group); or 7 days per week (continuous CAF group). Total days of access to CAF diet were matched between the latter three groups so that any group differences were attributable to the pattern of access. The continuous CAF and 5:2 groups had significantly more fat mass and worse short-term spatial recognition memory than the 3:4 and control groups. In Experiment 2, rats consuming CAF diet for 16 days and then returned to chow diet for 11 days displayed intact spatial memory, whereas those returned for 4 days were impaired, despite comparable reductions in adiposity in these two groups. CAF feeding did not impair object recognition memory in either experiment. These results demonstrate that the duration and pattern of access to CAF diet interact to determine effects on obesity and cognition in a graded fashion.

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The effect of *ACE* I/D polymorphism on the expression of fatty acid oxidation-related genes in human skeletal muscle after a single session of high-intensity interval exercise

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High-intensity interval exercise (HIIE) is associated with increased fatty acid oxidation, and the majority of fatty acids in skeletal muscle is stored in a form of intramuscular triglycerides (IMTG). The common *ACE* I/D gene polymorphism has been associated with exercise performance, with the I allele more prevalent in endurance-type athletes, who often have more IMTG and more fatty acid oxidation during exercise. We therefore hypothesise that the *ACE* I/D variant is associated with key genes controlling IMTG oxidation.

We analysed sixteen moderately-trained Caucasian males (age = 30.2 ± 7.9) from the Gene Smart cohort (Yan et al., 2017), ten homozygous for the D allele (*ACE DD*), and six homozygous for the I allele (*ACE* II). A graded exercise test (GXT) was used to assess peak power (W_{peak}) and lactate threshold (LT), both key endurance exercise phenotypes. A single session of HIIE (8 2-min intervals with 1 min rest between intervals) was completed by each participant. A muscle biopsy was taken before, immediately after, and 3 hours post HIIE from the *vastus lateralis* muscle and analysed for the expression of PGC1α, PPARα and PPARδ.

The expression of PGC1α was not different between the DD and II individuals at baseline (0.91 ± 0.25 vs 0.71 ± 0.09, $p = 0.08$). There was no difference in PPARα (1.40 ± 0.55 vs 1.90 ± 0.48, $p = 0.09$) or PPARδ (0.82 ± 0.35 vs 0.60 ± 0.11, $p = 0.17$). Three hours post HIIE, the expression of PGC1α, PPARα, PPARδ all increased (by 4.63 ± 2.30, 5.61 ± 3.40, 1.95 ± 1.18 fold, respectively, $p < 0.05$). However, the increase did not depend on *ACE* I/D gene polymorphism (4.11 ± 2.57 vs 5.49 ± 1.57, 5.67 ± 4.22 vs 5.49 ± 1.66, 1.92 ± 1.42 vs 1.99 ± 0.74 between DD and II individuals).

These preliminary results indicate a single session of HIIE significantly increased the expression of PGC1α, PPARα and PPARδ. However, this increase was not associated with the *ACE* I/D genotype. We are currently increasing the sample size to confirm these preliminary findings.

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Hepatocyte-specific deletion of Nox4 induces whole-body insulin resistance

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It has previously been shown that hepatic deletion of NADPH oxidase 4 (Nox4) exacerbates the effects of high-fat feeding. In particular, hepatic Nox4 deletion increases adiposity, impairs hepatic insulin signalling *ex vivo*, and also promotes glucose intolerance (via a glucose tolerance test) and impaired insulin sensitivity (via an insulin tolerance test). Here, we examined in more detail the cause(s) of insulin resistance *in vivo*, and hypothesised that hepatic Nox4 deletion would result in whole-body insulin resistance specifically due to impairments in hepatic insulin sensitivity. To test this, male mice with hepatic specific deletion of Nox4 (AlbCre-$Nox4^{fl/fl}$) and wild-type littermates ($Nox4^{fl/fl}$) were fed a high-fat diet (~23% energy from fat) for 12 wk, beginning at 6–7 wk of age. At 18–19 wk of age, mice had catheters placed into the left carotid artery and right jugular vein, and 5d post-surgery a hyperinsulinaemic-euglycaemic clamp was performed at two insulin doses (2.5 and 4 mU/kg/min) in conscious and unrestrained mice ($n = 6$–9 per group). At an insulin infusion rate of 2.5mU/kg/min, glucose infusion rate (GIR), endogenous glucose appearance (endoR_a), and insulin-stimulated glucose disposal rate (IS-GDR) were similar between AlbCre-$Nox4^{fl/fl}$ and $Nox4^{fl/fl}$ mice. In $Nox4^{fl/fl}$ mice, increasing the dose of insulin to 4 mU/kg/min increased GIR (22 ± 2 vs. 11 ± 1 mg/kg/min for 2.5mU/kg/min insulin, $p < 0.001$), enhanced the insulin-induced suppression of endoR_a (75 ± 12 vs. 40 ± 7%, $p < 0.05$), and augmented IS-GDR (12 ± 1 vs. 5 ± 1 mg/kg/min, $p < 0.01$). In contrast, increasing the dose of insulin from 2.5 to 4 mU/kg/min in AlbCre-$Nox4^{fl/fl}$ mice failed to alter GIR (12 ± 1 vs. 11 ± 1 mg/kg/min), an effect that was due to (a) an inability to further increase the suppression of endoR_a (53 ± 6 vs. 53 ± 3%), and (b) a failure to increase IS-GDR (5 ± 1 vs. 3 ± 1 mg/kg/min). Thus, AlbCre-$Nox4^{fl/fl}$ mice are insulin resistant. Furthermore, our findings indicate that the insulin resistance in these mice is due to hepatic and peripheral effects.

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