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Potential mechanisms of skeletal muscle insulin resistance in women with PCOS: a pilot study

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting metabolic, reproductive and mental health. It has a prevalence of 12–21% in Australian women and is associated with increased risk of insulin resistance (IR), gestational diabetes, early onset type 2 diabetes (T2D), cardiovascular disease, stroke and obesity. IR is present in 85% of women with PCOS and is mechanistically linked to dysfunctional skeletal muscle, a major contributor to whole body glucose uptake. However, unlike in other obesity related diseases, little is known about which molecular mechanisms in skeletal muscle contribute to the aetiology of PCOS. Transforming Growth Factor (TGFβ) ligand has been linked to an impaired improvement of skeletal muscle insulin sensitivity in T2D patients following an exercise intervention. Furthermore, TGFβ has also been associated with increased fibrosis in reproductive tissue of women with PCOS, predisposing them to metabolic and reproductive dysfunction. In this pilot study, we have investigated the effects of TGFβ and the Anti-Müllerian hormone (AMH), a novel TGFβ superfamily ligand elevated in serum of women with PCOS, on cultured myotubes from 20 overweight women with PCOS and 10 lean healthy controls, as a causal factor of skeletal muscle IR in PCOS. Our preliminary data show that the treatment with TGFβ and especially with high levels of AMH (30 ng/ml) on myotubes from overweight women with PCOS negatively affects insulin signalling and glucose uptake via TGFβ signalling pathway compared to healthy lean controls. This suggests that these TGFβ superfamily ligands may play a role in the skeletal muscle IR in PCOS. These results contribute to a better understanding of the aetiology of IR in PCOS but still further investigation is warranted to determine how these factors interact with other clinical markers.

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Expression of nutrient sensing mechanisms in the mouse stomach

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Background and aim: The ability of the gastrointestinal tract to detect nutrients is critical for the regulation of a range of physiological responses, including digestion and food intake. The detection of nutrients typically involves the interaction of a nutrient with a particular chemosensor. Limited information is available on the nutrient sensing capability of the stomach, with most of the studies primarily focusing the small intestine. Therefore, we investigated expression of nutrient sensors in the mouse stomach.

Methods: QRT-PCR was performed to investigate expression of nutrient sensors within the glandular regions (corpus and antrum) of the stomach of 8 week old male C57BL/6 mice. Duodenal samples were included as reference tissue. Target genes included sensors for the detection of protein digestion products (GPR93, CaSR and MGluR4), fatty acids (FFAR2, FFAR4 and CD36), the sweet and umami taste receptor subunit (T1R3), and cellular components involved in the transduction of the nutrient sensing signal (GNAT2, GNAT3 and TRPM5). Housekeeper genes were β2-microglobulin, peptidylprolyl isomerase A and hypoxanthine guanine phosphoribosyltransferase.

Results: Within the stomach, CaSR, GPR93, FFAR2, FFAR4, T1R3, TRPM5, and GNAT3 were identified with higher mRNA expression observed in the antrum than corpus. CD36 and GNAT2 were also present, with comparable expression in the antrum and corpus. No significant differences were observed between the antrum and duodenum for GPR93, MGluR4, FFAR4, T1R3 and TRPM5. Moreover, the expression of CaSR, FFAR2, GNAT3 and CD36 was higher in the corpus and antrum compared with the duodenum.

Conclusion: The mouse stomach expresses the genetic tools for the detection of nutrients, especially the digestion products of protein and fat. Furthermore, the level of expression is region-specific, with most targets presenting higher mRNA expression in the antrum than corpus. These gastric chemosensors may play a role in the modulation of gastrointestinal motility, digestive secretions and food intake.

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The impact of acute and chronic L-isoleucine supplementation on body weight and glucose tolerance in standard and high fat diet induced-obese mice

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Background: Dietary consumption of branched chain amino acids, including L-isoleucine, has been inversely associated with obesity in humans [1]. Acute L-isoleucine supplementation in standard laboratory diet (SLD) and high fat diet (HFD) fed rodents has been demonstrated to improve glucose tolerance, however, the effect of chronic isoleucine supplementation on glucose tolerance is unknown [2]. Therefore, we aimed to investigate the effect of chronic L-isoleucine supplementation on body weight and glucose tolerance in SLD and HFD-induced obese mice.

Methods: Male C57BL/6 mice (n = 60,8wks old) housed in a 12 h light-dark cycle were allocated into 2 groups and fed either a SLD (14%kJ from fat) or a HFD (60%kJ from fat) for 17 wks. After 12 wks, mice were allocated into 3 groups/diet (n = 10/group) and provided either normal drinking water (Control(C) & Acute(A)) or drinking water supplemented with 1.5% L-isoleucine (Chronic(Ch)) for the remaining 4 wks. At 17 wks, fasted mice received an oral gavage (0.1 ml/10 g body weight (BW)) of either normal water (C&Ch) or 300 mg/kg L-isoleucine (A) 30 min prior to an oral glucose tolerance test (OGTT; 1 g/kg BW).

Results: At 12 wks, HFD mice had gained more weight than SLD mice (SLD: 7.99 ± 0.34 g, HFD: 15.55 ± 1.67 g; unpaired-t-test (P = 0.0002). Between 13&16 wks, HFD mice gained more weight than SLD mice but there was no difference in weight gain within

dietary groups despite L-isoleucine treatment (SLD:C,1.58 ± 0.26 g; A,2.40 ± 0.25 g; Ch,2.49 ± 0.34 g; HFD:C,3.19 ± 0.44 g; A,4.59 ± 0.44 g; Ch,4.44 ± 0.85 g; 2 way-ANOVA ($P < 0.0001$) Dietary-effect). At 17 wks, fasting blood glucose was elevated in HFD mice compared to SLD mice but there was no L-isoleucine effect (SLD:C,10.84 ± 0.50 mmol/l; A,10.50 ± 0.31 mmol/l; Ch,11.15 ± 0.48 mmol/l; HFD:C,12.61 ± 0.60 mmol/l; A,12.90 ± 0.70 mmol/L; Ch,12.81 ± 0.66 mmol/l;2way-ANOVA ($P < 0.000$) Dietary-effect). The OGTT blood glucose area under the curve was elevated in HFD compared to SLD mice but there was no L-isoleucine effect within dietary groups (SLD:C,1651 ± 109.0 mmol/l-min; A,1760 ± 95.81 mmol/l-min; Ch,1980 ± 170.8 mmol/l-min; HFD:C, 2228 ± 174.1 mmol/l-min; A,2162 ± 191 mmol/l-min; Ch,2416 ± 232 mmol/l-min;2way-ANOVA ($P = 0.0022$) Dietary-effect).

Conclusion: Acute and chronic supplementation with L-isoleucine had no effect on body weight gain or glucose tolerance in SLD mice or HFD induced-obese mice.

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Obesity during pregnancy in rats adversely influences brain function in the offspring as adults

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Obesity is a low-grade inflammatory state and a fetus developing in such an inflammatory milieu is susceptible to developing various disorders. After birth, children of obese mothers are at increased risk of obesity, metabolic syndrome and neurodevelopmental abnormalities, eg reduced cognitive capacity, developmental delay, attention deficit hyperactivity disorder schizophrenia, and autism spectrum disorders. Here we used rats to test the effects of maternal obesity on neuronal networking and to determine if establishing a good diet at weaning could rescue deficits.

Rats gestated and maintained on an obesogenic Western diet (WD) had impaired spatial and working memory compared with rats on control chow (CC) at 10 weeks of age. Switching to CC when 3 weeks old completely rescued working memory but had no effect on spatial memory. Brain slices from 15 week old WD male rats had hippocampal epileptiform activity, which reflected current diet. This was supported by epileptiform spike-wave bursts, associated with a "freezing" of movement (absence), in EEG recordings in conscious free-moving animals. Immunohistochemistry revealed a marked reduction in GABA staining, with a reduction in NPY and somatostatin neurons reflecting current diet and somatostatin neurons also very much reduced in rats exposed to WD during gestation and weaning.

Activators of NPY receptors suppress spike-wave activity in absence epilepsies in humans and animal models and the reduction in NPY and occurrence of epileptiform activity in our WD rats supports these observations. A 2017 study of 1.4 million children from Sweden found that the incidence of childhood epilepsy increased with maternal BMI during pregnancy. Our results provide some insights into interpretation of this. Abnormal network and epileptiform activity has been associated with the early stages of Alzheimer's disease. It is alarming to consider that exposure to WD effects as early as *in utero* might predispose to earlier AD.

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Non-humanoid primate model of diet-induced obesity: model validation for biomarker discovery

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Obesity is a leading world health problem. Excess body weight is a major risk factor for developing type 2 diabetes, cardiovascular disease, dyslipidemia and other co-morbidities that ultimately lead to increased mortality rates. While pharmacotherapies can be employed to better manage obesity, they are hindered by their temporary effectiveness and side effects. This highlights the need for a better understanding of the pathophysiological features of human obesity. Non-human primates (NHPs) provide the most suitable pre-clinical model to investigate human obesity and discover novel therapeutic biomarkers. We have generated a high-fat diet (HFD)-induced NHP model of obesity and pre-diabetes in 34 pigtail macaques (*Macaca nemestrina*) consisting of 10 HFD males and 10 HFD females with 8 control males and 6 control females. Body composition analysis, glucose tolerance tests in addition to biopsy (muscle, fat and liver) and blood collections were completed at baseline and then every 3 months for 18 months in all animals. Both male and female HFD-fed animals displayed progressional increases in body weight and total body fat mass compared to baseline or lean controls that was due to largely adipocyte hypertrophy. Both HFD-males and HFD-females also demonstrated significantly increased fasting insulin concentrations and insulin area under the curve (AUC) compared to baseline or lean control counterparts determined via intravenous glucose tolerance tests. These findings suggest HFD-induced changes in insulin-sensitivity and development of insulin resistance at the end of the 18-month study. Obesity-induced changes in liver, muscle and fat morphology (steatosis, fibrosis, immune cell infiltration), circulating lipids (triglycerides, total cholesterol, HDL/LDL), metabolic hormones (leptin, adiponectin and C-peptide) and inflammatory markers (TNF-α, IL-1β, IL-6 and IL-10) are simultaneously being investigated using multiplex assays to further validate our model and provide the basis for future biomarker discovery studies.

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