

Ethical requirements of The Physiological Society

Experiments on animals or animal tissue

For work conducted in the UK all procedures must conform with current UK legislation. For work conducted elsewhere all procedures must accord with current national guidelines or, in their absence, with current local guidelines.

Experiments on humans or human tissue

All procedures must accord with the ethical standards of the relevant national, institutional or other body responsible for human research and experimentation, and with the principles of the World Medical Association's Declaration of Helsinki.



Abstracts

Plenary Lectures	44
Symposia	48
Poster Communications	70
Author Index	156

PL001

Bayliss & Starlings' gut hormones – curing the obesity pandemic

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Bayliss and Starling identified the “hormone” by reference to a gut substance released into the blood stream and acting at a distance. The intestine was subsequently found to have a range of endocrine cells in its mucosa which release peptide hormones into the circulation. They are stimulated both by luminal nutrients and by their basal innervation from the submucous neural plexus. They have a variety of digestive functions, eg gastrin stimulates gastric acid secretion, motilin enhances gut contractility, cholecystokinin triggers gall bladder contraction and pancreatic enzyme secretion. Secretin stimulates neutralising alkaline juice flow from the pancreas. Secretin is a member of much larger peptide hormone family. Another gut hormone member is Glucagon-like peptide (GLP1) which enhances insulin release and has recently triggered a new class of therapeutics for diabetes. What took longer to identify was that several of these gut hormones had wider actions, for example affecting hedonistic brain circuits to regulate appetite. The only successful therapy for obesity is surgical. The roux en Y gastric bypass procedure, for example, results in life long weight reduction, improved life expectancy, halving of cancer rates and complete remission of diabetes, amongst several other beneficial effects. How does it work? Initially thought to produce malabsorption, it has now been shown to work mainly through reduction of appetite. How does it do this? Elevation of the satiety inducing gut hormones, oxyntomodulin, PYY and GLP1 are thought key. Could these hormones be administered therapeutically – a sort of medical bypass? The Imperial team thinks they could and has spent five years successfully developing hormone preparations that can be administered once a week and which result in long term appetite reduction.

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PL002

Obesity - the Goldilocks paradigm

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Adipose tissue was once considered a simple structure whose sole function was to store excess energy as fat. This view has changed radically in the recent past as it has been found to have additional and complex functions. Specifically, it has been shown to function as an endocrine tissue releasing adipokines that signal

Plenary Lectures

to other tissues, and as an inflammatory tissue releasing cytokines and related molecules. In this talk, factors regulating adipocyte number and function will be reviewed, and how these are altered in the obese state and contribute to the adverse health outcomes linked to obesity. The concept of metabolically healthy obesity will be considered, together with the recognition that too little adipose tissue can be just as bad as too much from the perspective of adverse health and increased disease risk.

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PL003

Energy expenditure and energy intake – a question of balance?

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Energy Balance (EB) is widely believed to be a central concept underlying the development and maintenance of obesity. However EB should not be conceived as a simple mechanical balance with the difference between two values translated into weight loss or gain. This static model of EB is inappropriate and the widely used rule of thumb of 8000 kcal = 1 kg body weight is false and leads to misleading expectations of weight change. The dynamic model (Hall et al, 2011; Thomas et al, 2013) provides more realistic estimates of body weight change. EB is not just a case of energy in, energy out.

Ideas proposed more than 50 years ago (Edholm, 1955; 1970) but neglected until recently have led to renewed interest in the relation between EE and EI and to the interaction between physiology and behaviour. Behavioural actions (physical activity and eating) make huge contributions to EB. Edholm's proposal that 'the differences in intakes of food must originate in the differences in energy expenditure (Edholm et al, 1955 p 297) suggested a mechanism for the interaction between physiology and behaviour and for the role of EE in regulating appetite (EI). It is relevant that overfeeding (EI) influences EE in the form of spontaneous physical activity (Non Exercise Activity Thermogenesis – NEAT; Levine et al, 1999). Similarly, physical activity has the potential not only to influence EE but also to adjust EI. This perspective prompts a number of questions: does sedentariness downregulate EI to prevent a positive EB? Does physical activity upregulate EI in order to offset a negative EB? This raises the key issue of whether the physiological system compensates by increasing food intake when people engage in physical activity, thereby mitigating the impact of EE on weight loss.

Using a multi-level systems approach it can be demonstrated that persistent daily physical activity (exercise) selectively adjusts body composition and influences components of appetite control (King et al, 2009). Moreover the two major aspects

Plenary Lectures

of body composition (fat mass and fat-free mass) appear to play distinct roles in appetite control with fat-free mass being a driver of meals and total daily EI (Blundell et al, 2011; 2012). A mediating role for resting metabolic rate in EI (Caudwell et al, 2013) has supported Edholm's view that EI is related to EE. However when EE is adjusted by physical activity there is considerable individual variability in the outcomes indicating that the average response can be misleading. In principle, interactions between EE and EI can be mediated by adjustments in body composition, resting metabolic rate, substrate oxidation and by levels of tonic and episodic peptides influencing appetite. EE and EI is more than a simple question of balance.

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PL004

Are your genes to blame when your jeans don't fit?

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The recent rapid increase in obesity is undoubtedly due to changes in our lifestyle and in the types of food we eat. Our environment has changed dramatically over the past 50 years, with people taking much less exercise and consuming far more calories than ever before. However, although we are all exposed to these changes, not all of us are obese. Differences in our genetic make-up mean we all respond differently to the same environment. In fact, studies on twins have estimated the 'heritability' of body-weight to be anywhere between 40% – 70%. The fact that body weight is a highly heritable trait provides us the opportunity to use genetics as a tool to understand the molecular mechanisms underlying human obesity, thus teaching us about the biology of appetite control.

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PL005

Reversing type 2 diabetes to normal: The impact of the personal fat threshold

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What really causes type 2 diabetes? The best available information suggests that onset of type 2 diabetes is determined by relatively sudden failure of the beta cell to respond normally to a rise in blood glucose. Once established, the disease seems

Plenary Lectures

to behave as inevitably progressive with an irreversible beta cell defect. Certainly all the large studies of type 2 diabetes show a dismally progressive pattern such that around 50% of people require insulin therapy within 10 years. A variety of disease processes involving the pancreatic islets have been proposed to account for this, including amyloid deposition, oxidative stress and cytokine action. Where does insulin resistance fit in? It has seemed to be rather complicated.

Several recent pieces of information appear not to fit with this complex analysis. When people lose weight due to any cause, blood glucose levels return towards normal even when insulin or oral agents are stopped. By developing new methodology to measure fat content in specific organs it has been possible to answer these questions, and to demonstrate the basic simplicity of mechanisms underlying type 2 diabetes. Normal beta cell function can be restored by weight loss alone, and observation on individuals for up to 9 years confirms durability. The implications for people with diabetes are considerable.

However, this does not mean that type 2 diabetes is caused by obesity. Less than half of newly diagnosed people with type 2 diabetes have a BMI over 30kg/m², and around 1 in 7 have a normal BMI. But for any one individual it appears that there is a threshold effect. If a person has more fat on board than he or she can store safely in the inert subcutaneous depot then type 2 diabetes will develop irrespective of BMI. Understanding what happens in one person is not well described by the population metric of BMI.

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SA001

Obesity and its associations with cardiometabolic outcomes: Understanding the bigger picture

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This talk will set the scene for the four subsequent talks in the morning, and act as a general introduction for the entire three days. The pattern of BMI rise in developed nations will be shown along with a description of groups at particular risk of obesity or complications for given weight change. Several conditions linked to obesity but less well considered will also be briefly mentioned, for example, fatty liver disease, PCOS, pregnancy conditions and also some autoimmune conditions, to support need for obesity management in such areas. The need for better obesity management in diabetes patients will also be made. Of course, the obesity-cardiovascular link is commonly touted, but its causal role remains unclear with observational confusing J shaped curves. Similarly, BMI is not often taken into account in CVD risk scores, and why this is the case will also be explained. Finally, a brief discussion of the main drivers for obesity will be mentioned along with thoughts of what needs to be done by governments, health care professionals and public, to help stem the tide.

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SA002

Is obesity causally linked to CVD?

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Obesity (or adiposity more generally) has well characterised relationships with various intermediate measures of cardiovascular health and endpoints when assessed observationally. Whilst these have been derived from large and well undertaken studies, they of course suffer a series of known limitations which lead the community to need alternative approaches if interested in testing causality more directly. Trials of interventions reducing levels of adiposity, studies in differing confounding frameworks and prospective examination are all sensible approaches to dealing with this difficult problem, however to this has been recently added Mendelian randomisation. As part of a battery of methods used to help situations where causality is difficult to assess, the use of genetic variation reliably associated with a risk factor of interest (in this case obesity/adiposity/BMI) to act as a proxy measure for it can allow for the reassessment of causality and the generation of useful, clinically applicable, information. We have undertaken a series of studies across numerous data collections which have examined the causal impact of adiposity (measured via BMI) on cardiovascular outcomes and intermediates of cardiovascular health. Here I will summarise pertinent results and consider the implications of these for the consideration of adiposity as a risk factor and of BMI as a target for improving health.

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SA003

Effect of weight loss on type 2 diabetes

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Background: Type 2 diabetes is regarded as inevitably progressive with irreversible beta cell failure. However, resolution of type 2 diabetes has been possible with weight loss surgery, with evidence of restoration of normal metabolism. The hypothesis that both beta cell failure and insulin resistance that characterise type 2 diabetes can be reversed by dietary restriction of energy intake was tested.

Methods: Eleven people with type 2 diabetes (49.5 ± 2.5 years, BMI 33.6 ± 1.2 , nine male and two female) were studied before and after 1, 4 and 8 weeks of a 2.5 MJ (600 kcal)/day diet. Basal hepatic glucose output, hepatic and peripheral insulin sensitivity and beta cell function were measured. Pancreas and liver triglyceride

Symposia

content was measured using a three-point Dixon magnetic resonance imaging. An age-, sex- and weight- matched group of eight non-diabetic subjects was also studied. Results: After 1 week of restricted energy intake, fasting plasma glucose normalised in the diabetes group (9.2 ± 0.4 to 5.9 ± 0.4 mmol/l; $p = 0.003$) and remained in the normal range for the rest of the study duration. Fasting plasma insulin fell from 151 ± 104 to 73 ± 34 pmol/l after 1 week ($p = 0.03$) and to 57 ± 34 pmol/l by 8 weeks ($p = 0.03$ vs. baseline; $p = 0.04$ vs. non-diabetic group). Hepatic insulin sensitivity, assessed by the suppression of hepatic glucose production by insulin infusion, improved from $43 \pm 4\%$ to $74 \pm 5\%$ in the diabetes group ($p = 0.003$ vs. baseline; controls $68 \pm 5\%$). This was associated with 30% reduction in hepatic triglyceride content during the first week of energy restriction ($p < 0.001$). Hepatic triglyceride content continued to decrease and fell by 70% over the 8 weeks ($p = 0.003$). There was no change in peripheral insulin sensitivity expressed as glucose disposal rates during the entire study. After 1 week of acute energy restriction, fasting insulin secretion rate fell from 0.10 ± 0.03 to 0.06 ± 0.03 nmol/min/m² ($p < 0.05$) and remained constant thereafter. The first-phase insulin response increased (0.19 ± 0.02 to 0.46 ± 0.07 nmol/min/m²; $p = 0.006$; vs. non-diabetic group 0.62 ± 0.15 nmol/min/m²; $p = 0.42$ at 8 weeks). Maximal insulin response became supra-normal at 8 weeks (1.37 ± 0.27 vs. controls 1.15 ± 0.18 nmol/min/m²). These changes were seen in association with a decreased in pancreatic triglyceride content ($8.0 \pm 1.6\%$ to $6.2 \pm 1.1\%$; $p = 0.03$ vs. 8 weeks).

Conclusion: Normalisation of both beta cell function and hepatic insulin sensitivity in type 2 diabetes was achieved by dietary energy restriction alone. This was associated with decreased pancreatic and liver triacylglyceride stores. The twin defects of beta cell failure and insulin resistance that underlies type 2 diabetes are reversible by reducing dietary energy intake.

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SA004

Effect of weight loss on cardiovascular disease

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The obesity epidemic has the ability to undo the work of statins and smoking cessation on the prevalence of cardiovascular disease. It is well accepted that obesity is associated with an increased risk of cardiovascular disease and as such weight loss is often suggested to prevent cardiovascular events. Is this increased cardiovascular risk simply related to increased prevalence of adverse cardiovascular risk factors, such as hyperlipidaemia and hypertension, or are there adipose tissue specific factors that add to this effect? There is also conflicting evidence that once coronary heart disease is established, obesity appears to be protective. Achieving

Symposia

meaningful weight loss in individuals is difficult with only a fraction of patients even losing 5kg, which has led to difficulties in evaluating the effects of weight loss on cardiovascular events. This presentation will discuss the relationship between obesity and cardiovascular disease and the obesity paradox in established cardiovascular disease. The effects of weight loss on cardiovascular risk factors, surrogate markers and cardiovascular events will be presented, highlighting the gaps in the current understanding of this important area.

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SA005

Early life programming by parental obesity: A role for epigenetic pathways

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The ability of living organisms to respond to early nutritional exposures, and to evoke health-related effects in the next generation(s) has led to a growing interest in the “Developmental Origins of Health and Disease” (DOHaD) theory. Whereas maternal contributions are widely examined, effects of the paternal environment are often not considered. Several animal studies and a few epidemiological investigations on parental exposures to malnutrition and obesity indicate that transgenerational inheritance of environmentally induced functional changes of the genome, and related disorders, are driven by epigenetic components. We open the discussion on the existence of epigenetic windows of susceptibility to environmental insults during development of the male germ line. Changes in DNA methylation are viable mechanistic candidates for a non-genetic transfer of parental environmental information, from maturing germ cell to zygote. Inclusion of both, maternal and paternal nutritional factors, in future studies will ultimately improve the understanding of transgenerational epigenetic plasticity and health-related effects in future generations.

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SA006

New perspectives on the origin of hypertension; the role of the hypothalamic melanocortin system

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The growing epidemic of obesity has attracting increasing interest in the effect of obesity in pregnancy on the developing child. Independent associations have been reported in mother-child cohort studies between maternal obesity and hypertension in the offspring. The mechanisms of early onset hypertension are however less clear. This review focuses on novel advances in our comprehension of sympathetic mediated hypertension. My college and I have previously shown that maternal obesity in rodents leads to sympathetically mediated hypertension in juvenile offspring prior to obesity. This was associated with an exaggerated leptin surge in early postnatal life. Increased leptin during critical periods of development is likely to contribute to the onset of hypertension by altered leptin sensitivity and dysregulation of the neurotropic action of leptin. Unpublished evidence also suggests that the central melanocortin system, including melanocortin 4 receptors, plays a key role in early origins of renal nerve activation and hypertension in offspring of obese rat dams. Renal nerve denervation in juvenile rats restores blood pressure and renal function in neonatal hyperleptineamic rats. The origin of increase renal sympathetic overactivity is unknown but may include central action of renin angiotensin system and melanocortin system in brains regions involved in cardiovascular regulation. Other suggests that peripheral effects including renal ischemia can lead to sympathetic overactivation via central feedforward system. At a time when maternal obesity is reaching epidemic proportions, it appears crucial to better understand the adverse biological processes that mediate the origin of metabolic and cardiovascular dysfunction in the offspring. Recent studies indicate that maternal obesity can permanently influence the leptin-melanocortin system signalling in the offspring and therefore could represent a key mechanism for effecting long-term effects on appetite, renal sympathetic nerve activity and blood pressure.

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SA007

Developmental programming by maternal obesity: Mechanistic evidence from primate, sheep and rodent models

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Whilst the early postnatal leptin peak (between postnatal day 8 and 21) in rodents that programs the balance of orexigenic and anorexigenic appetitive neuropeptides and influences future leptin sensitivity is firmly established, existence and timing of a leptin peak in precocial animals (sheep and primates including humans) has received less attention. We have demonstrated a neonatal leptin peak between day 4 and 9 in sheep.

In obese sheep (OB) we showed that the neonatal leptin peak is eliminated in the first (F1) (Fig 1) and second generation (F2 - fed normal diet from weaning through gestation) offspring of OB ewes (Fig 1). Adult OBF1 and OBF2 offspring of OB mothers exhibited markedly increased appetites, glucose and insulin dysregulation, increased adiposity, and were hyperleptinemic compared to F1 and F2 offspring of control-fed F0 ewes.

OBF1 lambs and their offspring OBF2 lambs show elevated plasma cortisol at birth compared with control F1 and F2 lambs (Fig 1B and D). Cortisol has important roles in prenatal regulation of cell proliferation and differentiation to mature fetal tissues in preparation for extra-uterine life. Cortisol Levels higher than appropriate for current maturation may cause premature adipocyte differentiation, altering timing of the neonatal leptin peak. This hypothesis is supported by our findings that offspring of ewes given exogenous glucocorticoids in late gestation exhibited elimination of the neonatal leptin peak as seen in OB offspring.

Similarities and differences in maternal, fetal and perinatal life between rodents, sheep, nonhuman primates and man will be discussed.

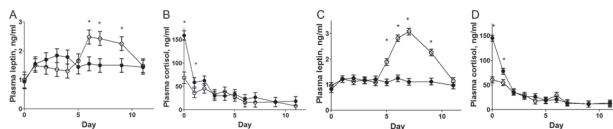


Fig 1. Neonatal plasma leptin A) F1 and C) F2 and cortisol B) F1 and D) F2 lambs of OB F0 (full circles) and control F0 ewes (open). M +SEM. * $p < 0.05$ OB vs. control; $n = 5-7$.

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SA008

Brain responses to food and weight loss

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In this symposium we will examine how functional neuroimaging has revolutionised the study of human eating behaviour. In the last 20 years, functional magnetic resonance and positron emission tomography paradigms have enabled researchers to understand how the human brain regions that control homeostatic and hedonic eating respond to food in physiological and pathological states. Hypothalamic, brainstem, limbic and cortical brain areas form part of a well-coordinated brain system that responds to central and peripheral neuronal, hormonal and nutrient signals. Even under physiological conditions it promotes the consumption of energy-dense food as this is advantageous in evolutionary terms. Its function is dysregulated in the context of obesity so as to promote weight gain and resist weight loss. Pharmacological and bariatric surgical interventions might be more successful than lifestyle interventions in inducing weight loss and maintenance, as unlike dieting, they reduce not only hunger but also the reward value of food through their actions in homeostatic and hedonic brain regions. Functional neuroimaging is a research tool that cannot be used in isolation; its findings become meaningful and useful only when combined with data from direct measures of eating behaviour. The neuroimaging technology is continuously improving and is expected to contribute further to the in-depth understanding of the obesity phenotype and accelerate the development of more effective and safer treatments for the condition.

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SA009

Effects of food and weight loss surgery on brain reward systems

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Akin to addictive behaviours, alterations in dopaminergic and opioid pathways, involved in the expectancy, appraisal and receipt of food reward appear to be important in the development and maintenance of obesity. Several components of the reward system, including the striatal nucleus accumbens and caudate nucleus (key to dopaminergic reward conditioning, expectancy and motivation), amygdala (emotional responses to rewarding stimuli), anterior insula (integrating gustatory and other sensory information) and OFC (reward value appraisal, cognitive control and attention) have been implicated. Activation in these areas to food cues not

only predicts food consumption and choice, and prospective weight gain, but may be altered in obesity, predict the success of weight loss strategies, changes with successful weight loss, including surgical treatments, and is altered in specific eating behaviour psychopathology such as dietary restraint, dietary disinhibition, binge eating and hyperphagia in genetic obesity. Interestingly modulation of activation of these reward systems both at rest and in response to food stimuli by gut hormones has been described.

Functional MRI offers a validated method of testing the effects of different bariatric surgeries on brain reward and cognitive control systems. Preliminary data from longitudinal fMRI studies suggest that RYGB may have beneficial effects on food hedonics including brain food reward systems, which may favour increased weight loss over BAND surgery. The differential effects of RYGB and BAND on brain food reward systems has not been tested, nor the relationship of these with behavioural and metabolic phenotypes (including exaggerated gut hormone release in RYGB) found in these two surgeries for obesity. The differential effects of RYGB and BAND surgery on brain structure including grey matter volume and white matter tract integrity has also not been examined. Understanding how different surgeries differentially affect eating behaviour and food reward on a functional and anatomical level in the brain may help establish the mechanism by which RYGB achieves greater success in treating obesity. This not only highlights the importance of gut-brain food hedonics in the treatment of obesity, including the development of novel, non-surgical treatments, but also raises the potential of more personalized approaches to surgical obesity treatments, according to relevant clinical, behavioural and metabolic phenotypes.

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SA010

The role of CNS interleukin-6 (IL-6) and IL-1 in tonic suppression of body fat during health

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Early data obtained in gene knockout mice indicate that the cytokines IL-6 (1, 2) and interleukin 1 β (IL-1 β) (3, 4) exert a tonic anti-obesity effect in the CNS, an effect that partly may be due to stimulated thermogenesis and enhanced leptin sensitivity. Therefore, IL-6 and IL-1 seem to exert metabolic effects during health in addition to modulating the immune response during disease. Glucagon-like-peptide-1 (GLP-1) is produced by L-cells in the intestine and in the brainstem. GLP-1 analogues are used clinically to enhance insulin secretion from the pancreas, and this way improve glucose metabolism in type 2 diabetes. GLP-1 and its analogues also reduce food intake and body weight, clinically beneficial actions that are likely exerted at

Symposia

the level of the CNS. Recently, we found that central injection of a clinically used GLP-1 receptor agonist, exendin-4, potently increased the expression of IL-6 in the hypothalamus (11-fold), the caudal hindbrain (4-fold) and the parabrachial nucleus (PBN) of the pons. Exendin-4 increased IL-1 β expression in the hypothalamus, but not the brainstem or PBN. Exendin-4 treatment did not change the expression of, tumour necrosis factor-alpha TNF-alpha, the third classic pro-inflammatory cytokine, indicating that GLP-1 does not exert a general pro-inflammatory effect (5). Pharmacologic disruption of IL-1 or IL-6 biological activity attenuated anorexia and body weight loss induced by central exendin-4 administration. Simultaneous blockade of IL-1 and IL-6 activity led to a more potent attenuation of exendin-4 effects on food intake. Mice with global IL-1 receptor gene knockout or central IL-6 receptor knockdown showed inhibited decrease in food intake and body weight in response to peripheral exendin-4 treatment. In conclusion . the two cytokines IL-6 and IL-1 exert anti-obesity effect at the level of CNS. They mediate the body weight loss caused by central GLP-1 receptor activation

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SA011

Physiological links between circadian rhythms, metabolism and nutrition

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Circadian rhythms, metabolism and nutrition are closely inter-linked. A great deal of recent research has not only investigated how aspects of metabolic physiology are driven by circadian clocks, but how these circadian clocks are themselves sensitive to metabolic change. At the cellular level, novel feedback loops have been identified that couple circadian 'clock genes' and their proteins to expression of nuclear receptors, regulation of redox state and other major pathways. Using targeted disruption of circadian clocks, mouse models are providing novel insight into the role of tissue-specific clocks in glucose homeostasis and body weight regulation. The relationship between circadian rhythms and obesity appears complex, with variable alteration of rhythms in obese individuals. However, it is clear from animal studies that the timing and nutritional composition of meals can regulate

circadian rhythms, particularly in peripheral tissues. Translation of these findings to human physiology now represents an important goal. This seminar will highlight protocols for the study of human circadian rhythms and the development serial biopsy analysis. It will then demonstrate how these techniques can be utilised to investigate the interactions between human rhythms, obesity, type 2 diabetes and feeding time.

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SA012

AMP-activated protein kinase: Regulating energy balance at both the cellular and whole body levels

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The AMP-activated protein kinase (AMPK) is a critical sensor of energy status that appears to have arisen very early during the evolution of unicellular eukaryotes. Its cell-autonomous role in energy homeostasis is conserved in multicellular eukaryotes, but the role of AMPK also appears to have adapted so that it now regulates energy balance at the whole body level (Hardie & Ashford, 2014).

AMPK exists universally as heterotrimeric complexes comprising catalytic α subunits and regulatory β and γ subunits. The kinase activity increases >100-fold upon phosphorylation at Thr172 within the α subunit by upstream kinases, which include the tumour suppressor kinase LKB1 and the Ca^{2+} /calmodulin-dependent protein kinase, CaMKK β . Cellular energy stress caused either by inhibition of ATP synthesis (e.g. during hypoxia or hypoglycaemia) or by acceleration of ATP consumption (e.g. in muscle during exercise) leads to increases in the cellular ADP:ATP ratio, which are amplified by adenylate kinase into even larger increases in AMP:ATP. Displacement of ATP by AMP at multiple sites on the γ subunit leads to activation of AMPK by three complementary mechanisms: (i) promoting Thr172 phosphorylation by LKB1; (ii) inhibiting Thr172 dephosphorylation; (iii) triggering a substantial allosteric activation (Gowans et al., 2013). AMPK is activated by the major anti-diabetic drug metformin, which acts indirectly by causing inhibition of the respiratory chain and thus increasing cellular AMP (Hawley et al., 2010). AMPK is also directly activated (and phosphorylation of Thr172 promoted) by synthetic drugs that bind at a site located between the kinase domain on the α subunit and the carbohydrate-binding module on the β subunit. Ligands that bind this site that occur naturally in mammals have not yet been identified, although the plant product salicylate (the major breakdown product of aspirin) also binds here, causing activation of AMPK and perhaps explaining some therapeutic effects of aspirin (Hawley et al., 2012).

Once activated by a cellular stress that causes ATP depletion, AMPK acts to restore energy homeostasis by phosphorylating numerous downstream targets. These either switch on catabolic pathways generating ATP, or switch off processes consuming ATP, including lipid, polysaccharide and protein biosynthesis. For example, AMPK switches on fatty acid oxidation acutely by phosphorylating acetyl-CoA carboxylase-2 (ACC2) and thus lowering malonyl-CoA, and in the longer term by promoting mitochondrial biogenesis and expression of oxidative enzymes. At the same time, it switches off fatty acid synthesis acutely by phosphorylating and inactivating acetyl-CoA carboxylase-1 (ACC1), and in the longer term by inhibiting transcription of fatty acid synthesis enzymes. To examine the importance of the acute regulation of ACC, mice with double knock-in (DKI) substitutions that prevent their phosphorylation by AMPK (ACC1-S79A, ACC2-S212A) were generated (Fullerton et al., 2013). While not obese, these mice had elevated di- and tri-acylglycerol in liver and muscle, displayed signs of fatty liver, and became severely glucose-intolerant and insulin-resistant. These metabolic parameters deteriorated in wild type but not DKI mice that were fed a high-fat diet, so that they became similar to those of the DKI mice. However, while the metabolic parameters all improved when the wild type mice were treated with metformin, those of the DKI mice did not. These results suggest that the insulin-sensitizing effects of metformin are mediated entirely by phosphorylation of ACC1 and ACC2 by AMPK, with an associated reduction in lipid storage.

In addition to these cell-autonomous effects, the AMPK system also mediates energy balance at the whole body level, particularly via effects in the arcuate nucleus of the hypothalamus. The hormone ghrelin, a “hunger hormone” released from the gut during fasting, activates AMPK via GSHR2 receptors and the Ca^{2+} /CaMKK β pathway in presynaptic neurons upstream of agouti-related protein (AgRP) expressing neurons (Yang et al., 2011). This leads to activation of the AgRP neurons, causing an orexigenic effect. On the other hand, the “satiety” hormone leptin, released from adipose tissues that have adequate triacylglycerol stores, inhibits AMPK in the same neurons and thus causes an anorexigenic effect (Yang et al., 2011). It has been proposed that insulin and leptin (the latter most likely acting indirectly via release of an opioid such as β -endorphin) both inhibit AMPK in these neurons by activation of the PI 3-kinase-Akt-S6K1 pathway, with S6K1 phosphorylating AMPK at a site that inhibits its phosphorylation and activation by LKB1 (Hardie & Ashford, 2014; Hawley et al., 2014).

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Symposia

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SA013

Insulin action in the brain

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Insulin resistance is a hallmark feature of type 2 diabetes and obesity. In addition to the classical view that insulin resistance in the liver, muscle, and fat disrupt glucose homeostasis, studies in the past decade have illustrated that insulin resistance in the hypothalamus disregulates hepatic glucose production and food intake, leading to type 2 diabetes and obesity. My lecture argues that insulin signaling in the dorsal vagal complex regulates hepatic glucose production and food intake. A thorough understanding of the physiological and pathophysiological mechanisms of insulin action in the hypothalamus and dorsal vagal complex is necessary for identifying therapeutic targets for obesity and type 2 diabetes.

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SA014

Lifestyle interventions in obese pregnant women; do they work?

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Obesity in pregnancy increases the risk of complications for mother and child. The increasing prevalence of obesity amongst pregnant women has led to a global effort to develop interventions which are safe, and prevent development of gestational diabetes, pre-eclampsia, fetal macrosomia or delivery of a large for gestational age infant, amongst other adverse outcomes. Two approaches have been taken, pharmacological or a change in lifestyle. The focus of this talk will be on lifestyle interventions. To date, the majority have centred on recommendations which combine changes in diet and physical activity. Theoretically, these may be effective through reducing maternal insulin resistance which plays a causal role. Much of the focus has been towards restriction of gestational weight gain (GWG), a surrogate end point for clinical outcomes, as excessive GWG is associated with increased risk of complications. This approach, whilst negating the need for larger clinical trials, adequately powered for clinical outcomes, has several limitations. Meta-analysis of these studies has revealed that it is possible to achieve a modest reduction in GWG, but with little influence on clinical outcomes (Thangaratnam et al 2013). Some interventions have been underpinned with theoretical strategies designed to support behavioural change and others have not. Compliance to the intervention has infrequently been evaluated eg assessment of the change in diet or physical activity achieved. A recent systematic review of the methodologies used concluded that motivational interviewing and behavioural self monitoring are likely to be key factors in achieving limitation of GWG (Hill, Skouteris et al. 2013). The LIMIT trial was the first adequately powered lifestyle intervention RCT for obese and overweight pregnant women to address clinical endpoints (Dodd, Turnbull et al. 2014). Dietary advice incorporated food exchanges, increased fibre and fruit consumption and reduction of refined sugars. The primary outcome of a reduction in incidence of delivery of a large for gestational age infant was not met, but there was a reduction in risk for the secondary outcome of macrosomia in the intervention group (15% v 19%, RR 0.83, [95% CI 0.68 to 0.99], $p=0.04$). The UK UPBEAT randomised controlled trial (Poston et al, 2014) has recently finished recruitment, and will report in 2015. Here the intervention, more intense than most, focused on reducing the dietary glycemic load and increasing physical activity. In summary, there is to date no proven lifestyle intervention which can be recommended in the antenatal care of obese pregnant women.

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Symposia

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA015

Managing obesity in primary care and general practice

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More than a quarter of adults are now classified as obese and a further 42% of men and a third of women are overweight. The potentially preventable illness and premature death caused by obesity costs the NHS around £5.1 billion per year. Given the high prevalence of obesity there is a need to investigate the effectiveness of simple, cheap and pragmatic interventions that have the ability to be reach the high number of people needing to lose weight in the UK. Much of this management of obesity could potentially be initiated by GPs and primary care settings are the ideal place in which to intervene since most of the population can potentially be reached. Guidelines recommend that primary care physicians in England should identify people with obesity and offer clinical management, but few options for treatment exist in traditional primary care settings. Studies have shown GPs have a generally negative or ambivalent attitude to obesity management and they are seemingly reluctant to become involved. Studies have also reported GPs have a lack of confidence in the effectiveness of potential management options and limited time within a routine 10 min consultation to intervene. One potential option available to GPs that might address some of these issues is for GPs to refer their obese patients to widely available commercial weight management programmes (e.g. Slimming World and Weight Watchers) and in many areas in England the NHS offers these services free to patients and evidence supports the effectiveness of such provision. An alternative, potentially widely available, management option for obesity is for primary care teams to treat their obese patients themselves but recent evidence suggests they are ineffective and qualitative work has indicated that patients would prefer support from external agencies anyway.

We conducted the Lighten Up trial (Jolly et al 2011, BMJ) where we investigated the effectiveness of several pragmatic interventions in 740 obese or overweight adults with a co-morbid disorder identified from general practice records. Participants were randomised to entitlement to free commercial weight loss management programmes (Weight Watchers, Slimming World or Rosemary Conley), primary care management (general practice or pharmacy support), a choice of any programme and a minimal intervention comparator group for 12 weeks. All programmes achieved significant weight loss from baseline to programme end at 12 weeks follow up (range 1.37 kg (general practice) to 4.43 kg (Weight Watch-

Symposia

ers)), and all except general practice and pharmacy provision resulted in significant weight loss at one year. At one year, only the Weight Watchers group had significantly greater weight loss than the comparator group with 2.5 kg greater loss. Collectively the commercial programmes achieved significantly greater weight loss than did the primary care interventions at programme end (mean difference 2.3 kg). The primary care programmes were the most costly to provide. The results of the Lighten Up trial are similar to those of the trial by Jebb and colleagues (Lancet 2011) where 772 participants randomised to 12 months Weight Watchers or GP care achieved a weight loss of 4.0 kg and 1.6 kg respectively at 1 year follow up. Evidence indicates that commercially provided weight management services are more effective and cheaper than primary care based services, which are ineffective and not popular with patients. However, the vast majority of people who lose weight will regain this weight within 1-2 years therefore it is critical that effective interventions are also available to ensure weight loss is maintained over the longer term.

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SA016

Mechanisms linking maternal diet-induced obesity to offspring metabolic health - insight into potential intervention

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It is well established that events in fetal and neonatal life can influence an individual's risk of developing metabolic conditions such as type 2 diabetes, insulin resistance and cardiovascular disease in later life. Much of the early focus of such "programmed" events was directed towards the effects of maternal under-nutrition and low birth weight on the long-term health of an individual. However in light of the growing epidemic of obesity, including in women of child-bearing age, attention has now been directed towards understanding the effects of maternal over-nutrition during pregnancy and lactation on the long-term health of the offspring¹. To address this we, and others, have used a mouse model of maternal diet-induced obesity where pregnant and lactating mice are fed a diet rich in saturated fats and simple sugars (reflective of a human westernized diet) to induce obesity prior to pregnancy. This maternal dietary manipulation leads to cardiovascular dysfunction² and insulin resistance in the offspring that is associated with post-transcriptional programming of insulin signaling protein expression³. The offspring of obese dams are also more susceptible to diet induced obesity. As well as utilizing the mouse model as a tool to identify mechanisms through which programmed changes in the offspring arise, we have also utilized it to identify factors in the obese mother that mediate the detrimental effects in the offspring. Maternal

Symposia

hyperinsulinaemia, which is amenable to lifestyle interventions such as increased physical activity, has emerged as one important parameter. These findings in an animal model therefore provide important insight into rational intervention strategies that have the potential to prevent transmission of metabolic dysfunction between generations.

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SEO is a BHF Senior Fellow and a member of the University of Cambridge MRC Metabolic Diseases Unit

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SA017

Interactions between gut microbiota and intestinal host cells in metabolic disorders associated with obesity: an interesting target of intervention

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Obesity is associated with a cluster of metabolic comorbidities (type 2 diabetes, cardiovascular diseases, and non-alcoholic steatohepatitis). Identify novel targets with therapeutic potential would bring hope to control this epidemic disease and related metabolic disorders. Growing evidence supports the idea that the increased prevalence of obesity and type 2 diabetes cannot be attributed solely to changes in the human genome, nutritional habits, or the reduction of physical activity in our daily lives. Among other candidates, a specific environmental factor evolving with us from birth and our dietary habits has been shown to contribute to energy homeostasis, this is the gut microbiota

We have contributed to the demonstration that the gut microbiota composition is associated with several hallmarks of metabolic syndrome (e.g., obesity, type 2 diabetes, cardiovascular diseases, and non-alcoholic steatohepatitis). Unequivocal evidence demonstrates that gut microbiota influence whole-body metabolism by affecting the energy balance, gut permeability, serum lipopolysaccharides (i.e., metabolic endotoxemia), and metabolic inflammation that are associated with obesity and associated disorders.

Symposia

Because the gut microbiota is a crucial actor involved in the pathology of obesity and type 2 diabetes, gut microbiota modulations are viewed as an interesting tool to treat these diseases. In the 90's our lab has discovered and developed the prebiotic concept. Since this discovery, we have shown that, specific gut microbiota modulations using prebiotic (i.e., such as oligofructose a non-digestible carbohydrates) improve gut barrier functions, metabolic endotoxemia, inflammation, glucose and lipid homeostasis in obesity and type 2 diabetes. These targeted nutritional interventions using non-digestible carbohydrates with prebiotic properties have shown promising results in pre-clinical studies in the context of obesity; however the bacteria as well as the mechanisms of interaction between the gut microbiota and the host involved in these beneficial effects of gut microbiota modulations remain poorly understood.

Recently, we have revealed that cross-talk between a specific bacterium namely *Akkermansia muciniphila* and intestinal epithelium is able to reduce diet-induced obesity, type-2 diabetes and gut permeability as well as whole body inflammation associated with obesity. Although these results provide a rationale for the development of a treatment that uses this human mucus colonizer for the prevention or treatment of obesity and its associated metabolic disorders, this remain to be confirmed in human patients.

In conclusion targeting the gut microbiota in the context of obesity have shown promising therapeutically advance in pre-clinical studies, although human intervention studies warrant further investigations.

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SA018

The Healthy Lifestyles Programme (HeLP); evidence of feasibility, acceptability and proof of concept in affecting children's weight status

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Background: Despite the rise in childhood obesity there is still a paucity of evidence for effective interventions that engage children and parents sufficiently to make and sustain lifestyle behaviour change. Most approaches in schools use traditional techniques (lessons, additional activity classes) to affect behaviour change.

Objectives: To develop a drama-based, school-located obesity prevention programme, that considers both intervention components and the system in which the intervention is delivered and engages children, their families and schools such that supportive environments for behaviour change are created at home and within the school

Methods: HeLP was developed iteratively using evidence and behavioural theory with a complex systems framework. Three phases of piloting (398 children),

Symposia

involving extensive stakeholder consultation and including an exploratory trial have taken place and an RCT involving 1350 children is underway.

Results: Phase 1 and 2 identified the appropriate age group and enabled refinement of intervention messages, activities and modes of delivery. HeLP takes place over 3 school terms and involves four phases; creating a receptive context, the healthy lifestyles week, goal setting and reinforcement activities. In the exploratory trial (4 schools), 24 month measures (anthropometric and behavioural) were obtained from 92% of original cohort. Positive changes were seen in all targeted behaviours in the intervention group. In the control schools the proportion of children overweight/obese rose from 26% at baseline to 32% at 18 and 24 months while, for intervention schools, the proportion remained at baseline levels (24%).

Conclusion: HeLP is a dynamic Programme that has been designed to allow for local adaptively, it is feasible to deliver and engages schools, children and families. 'Proof of concept' has been established. Its effectiveness, cost effectiveness and an detailed process evaluation are being assessed in a cluster RCT which involves 32 schools and 1350 children.

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SA019

Public Health England: Working together to tackle and prevent obesity

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Public Health England (PHE) exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. PHE is responsible for supporting local authorities take action to tackle poor diets, inactivity and sedentary lifestyles and excess weight. PHE shares the ambition with the Department of Health to achieve a downward trend in excess weight among children and adults by 2020 and recognises that tackling obesity requires further action .

Obesity is a complex issue the root causes, of which are embedded within the environment we live in, the food supply, our behaviours, physiological, psycho-social factors and the impacts of health inequalities that exist across the population .

In England, poor diet and excess weight are risk factors for increased mortality and we know that obesity has a major impact on people's health increasing the risk of type 2 diabetes, hypertension and colorectal cancer . The prevalence of obesity, in England, remains serious with 22.2% of children by the age of 4 to 5 years overweight or obese, rising to 33.3% in children aged 10-11 years . There are clear social gradients in child obesity with the obesity rate in the most deprived 10% being approximately double that of the least deprived 10%. In the adult population 67% of men and 57% women are obese or overweight .

Symposia

PHE is taking forward a breadth of actions, across the life course, to support local action to tackle and prevent obesity. Building on dialogue with Directors of Public Health PHE has developed a work plan across five themes, namely systems leadership, community engagement, supporting delivery, monitoring and the evidence base and tackling the obesogenic environment.

PHE aims to support local authorities to develop early intervention and referral into weight management services through the National Child Measurement Programme and NHS Health Checks. PHE has a key role in enabling effective local practice relating to the development and evaluation of interventions and has a significant role in the translation of learning into useful information for local practitioners.

We also support action to make the healthier choice the easiest choice for people through for instance healthier food procurement and enabling behaviour change and healthier lifestyles, in families and communities through the Change4Life social marketing campaign and other targeted interventions.

PHE cannot achieve this in isolation and to achieve a shift in the population's weight, PHE needs to work across all levels of the health, public health and social care system investing in a diversity of partnerships, including community action, third sector, royal colleges and commercial organisations.

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Symposia

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SA020

Getting science to the citizen

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Public engagement is increasingly being recognised as an important activity for the UK and European scientific community. Through access to the scientists who are performing research on their behalf, the interested public can not only hear about the latest research and informed opinion but can obtain important context about where science contributes to, and is relevant to, their everyday life – health, economy, culture etc. The need for scientists to grasp engagement opportunities and present a perspective of science that goes beyond that available in the popular media is now widely recognised, and is a necessary output from research funded at UK national and EU levels. Whereas individual public engagement events may provide direct access to only a relatively small self-selected group of interested individuals, improving technology potentially allows everyone to access these interactions between scientists and the public. Positioning such resources where they can be found by the public will not only make science accessible to a wider audience but could also provide a useful supplement to more formal popular education platforms such as MOOC – online open access courses. A number of public engagement vehicles will be discussed, based on the dissemination activity undertaken as part of the EU-funded projects, Full4Health (www.full4health.eu), NeuroFAST (www.neurofast.eu) and SATIN (www.satin-satiety.eu), and the Scottish Government Strategic Research Programme, Healthy Safe Diets theme.

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SA021

‘Responsibility Deal’ or responsibility; what should companies really do to combat obesity?

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A recent suggestion made by Professor Susan Jebb to combat increasing obesity among children was to insist that water replaces soda, juice or cordials at mealtimes. The problems with this as a solution have been readily pointed out in

Symposia

the media. But above all and like so many recent suggestions the onus is on the individual to change their eating behaviour while living in culture that constantly exhorts them and their children to indulge in highly calorific foods.

At the time of writing the other most discussed remedy is to introduce a sugar tax. The arguments for targeted interventions to change eating habits through public information, taxes and regulation have been well documented elsewhere (Griffith, and O'Connell, 2010), what has received much less attention has been the relative responsibility of firms and individuals in tackling obesity.

The role of business in the obesity problem has been compared in the media to that of the tobacco industry not that long ago. Others suggest that business is not concerned as to whether the population gets fat or not rather 'what they care about is that we buy it from them [McDonalds] and not from Hardee's Wendy's or Jack in the Box' (Wansinck, 2010). This from a leading researcher in the field appears to expunge business of responsibility. The quote above indicates that the overriding concern of businesses is a competitive one; they are more concerned with shareholders than stakeholders, and if it sells (and it's legal) they will sell it to us. A time when every business that is a household name espouse their corporate social responsibility credentials, we should question why aren't these companies more concerned as to how much and what we eat?

The main attempt in the UK to engage with businesses has been through the current government's Responsibility Deal. The problem with such an initiative, however, is there is no overall incentive for firms to be producing healthier food with lower calories as a matter of course. There are individual initiatives but that is not going to change the substantial amount of attractive, cheap and calorific food in the average supermarket or convenience store. Meanwhile business strategies, tactics and innovation all seem to be pointing to encouraging us to consume more calories. Here are a few examples. Recently a major global food producer which is part of the Responsibility Deal promoted its well known chocolate bar which normally consists of four wafer fingers covered in chocolate in a limited edition of five fingers 'for sharing' – seems like four is easier to share than five. The favoured promotion in most supermarkets is buy two get one free, and also common now is buy three food types for a fixed price. In each case you may not need or want the additional quantity but the bargain offered encourages buying more. In his book 'Addicted to Food', James Erlichman describes another tactic used by food manufacturers, they undersize portions. All food must carry the contents and calories of 100 grams of a product. But manufacturers help us work out our calorie and nutrient content by also offering the contents of a typical portion size; the trouble is that our portion size is likely to be much larger than their 'undersized portions'. This paper will consider in more detail what responsibility means in both an individual and corporate sense and argue for a different set of pledges in any further Responsibility Deal.

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Symposia

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SA022

Obesogenic environments: Exploring food environments

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The food environment or the 'foodscape' has changed rapidly in the UK, and globally, over the last twenty years. Alongside this change has been an exponential increase in the prevalence of overweight and obesity. This has stimulated research across the world to explain the relationship between aspects of food retailing, diet and health.

In relation to obesity, environmental factors influence both sides of the energy balance equation; energy intake, in terms of the food environment and eating behaviours, and energy expenditure, describing physical activity and the environment. In terms of disease prevention and the promotion of health it is important to establish which aspects of the food and physical activity environments are amenable to change.

Research is increasingly focusing the interaction between nutrition behaviour and the environment. However this relationship between food choice, dietary habits and the environment is complex.

Previous work has tended to focus on food availability in the environment either in terms of the spatial distribution of food shops in relation to the socio-economic status of communities or looking at neighbourhood food availability and individual level food intake. There is a need to understand the mechanisms by which the environment influences individual food choice and dietary intake as well as exploring what factors within the environment are amenable to change.

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PC001

Body mass index influences the age at menarche and duration of menstrual cycle

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Early menarcheal age and menstrual irregularities are commonly associated with high body mass index (BMI)^{1,2}. Menarcheal age of 14 years and over has also been linked with prolong and irregular cycles³. The impact of BMI on the age at menarche and duration of the menstrual cycle in normal (NM), overweight (OW) and obese (OB) young school girls in south-south Nigeria was examined.

Participants (n = 52; median age = 20 years) were classified into 3 groups: NM (n = 27; BMI = 21.0 ± 2.0 Kg/m²), OW (n = 14; BMI = 28.1 ± 1.2 Kg/m²) and OB (n = 11; BMI = 31.5 ± 0.6 Kg/m²). BMI was calculated from the equation; BMI = weight (Kg)/height (m)²⁴. Cross-sectional data on menarche and menstrual cycle history were obtained through self-administered questionnaires. Approval and consent were obtained from the school authorities and participants before the study commenced.

Data analysis showed a statistical significant difference in the BMI ($p < 0.0001$). Though the mean (\pm SD) ages at menarche were similar (NM = 13.0 ± 1.2 years; OW = 12.9 ± 0.9 years; OB = 13.7 ± 0.9 years) ($p > 0.05$), the average duration of the menstrual cycle (NM = 27.3 ± 1.4 days; OW = 27.9 ± 0.6 days; OB = 29.4 ± 1.4 days) and menstrual bleeding (NM = 4.4 ± 0.6 days; OW = 4.4 ± 0.8 days; OB = 5.4 ± 0.9 days) differed significantly according to the BMI ($p < 0.0001$ and $p < 0.05$ respectively). There was an increase in the average length of the menstrual cycle with increased BMI ($r = 0.515$, 95% CI = $0.28 - 0.69$, $p < 0.0001$). However, the correlation between BMI and age at menarche was weak ($r = 0.2527$, 95% CI = $-0.02 - 0.49$, $p > 0.05$). The average duration of the menstrual cycle tend to decrease with older menarcheal age, though this was not statistically significant ($r = -0.016$, 95% CI = $-0.29 - 0.26$, $p > 0.05$).

A higher than normal BMI may be an important risk factor for longer menstrual cycles and menses (menstrual irregularities), with adverse consequences for future reproductive and overall health of young females.

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PC002

Nesfatin-1, an anorexigenic neuropeptide, affects pubertal maturation via kisspeptin/GPR54 system in the female rats

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There is a close relationship between energy homeostasis and reproductive functions. But, the molecules responsible for this interaction remain to be explored. Nesfatin-1, an hypothalamic neuropeptide, is suggested to be another candidate for the metabolic regulation of reproductive functions besides leptin, which has been shown to exert its effects on hypothalamic-pituitary-gonadal axis by means of kisspeptin/GPR54 system. Nesfatin-1 has an anorexigenic effect¹, and its circulating level is positively correlated with body mass index (BMI)². This study was carried out to investigate whether there is an interaction between nesfatin-1 and kisspeptin/GPR54 system in terms of pubertal maturation. For this aim, the pre-pubertal Sprague-Dawley female rats were weaned on day 21. They were intracerebroventricularly cannulated and injected with nesfatin-1 or peptide 234, a kisspeptin antagonist, in the dose of 25 and 50 pmol, daily. Cannulation was surgically performed under general anesthesia with ketamine 60 mg/kg plus xylazine (rompun) 5 mg/kg. Puberty onset was monitored by examination of vaginal opening (VO). Body weight was daily determined, and vaginal opening was daily monitored starting from day 26. The animals were decapitated from day 60 when diestrus, which was determined by vaginal smears, was observed. Nesfatin-1 advanced VO compared to sham rats (36 versus 38 days, respectively). In the rats given nesfatin-1 and peptide 234 together, puberty onset was similar to sham rats. Pubertal weight was found to be lower ($P<0.05$) in nesfatin-1 injected rats compared to sham rats (73.5 ± 2.17 and 86.9 ± 3.55 g, respectively). Body weight was lower ($P<0.003$) in the nesfatin-1-injected rats compared to sham rats at the end of the experiment. Body weight was similar in the rats given nesfatin-1 and peptide 234 together. In conclusion, the nesfatin-1 seems to affect pubertal maturation by means of kisspeptin/GPR54 system in the female rats. Since obesity is suggested to advance puberty onset, nesfatin-1 may be responsible for obesity-dependent pubertal maturation. Oh-I S *et al* (2006). *Nature* **443**, 709 –712.

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PC003

Repression of hepatic intracellular cholesterol transporters in obese Zucker (fa/fa) rats: Functional implications for dyslipidaemia and steatosis

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Excess adipose tissue associates with insulin resistance, high levels of triglyceride-rich VLDL, low levels of HDL and hepatic steatosis. The impact of obesity on lipid transport within the liver is unknown. This study investigated expression of cholesterol-trafficking proteins, mitochondrial StarD1 and endosomal StarD3, their relationship with dyslipidaemia and steatosis in genetically obese rats, and their functions in lipid metabolism in rat McArdle RH-7777 hepatoma cells.

Heterozygous Zucker rats (Fa/fa) were purchased from Harlan Laboratories (Bicester, UK). Male and female, lean and obese rats were terminally anaesthetized at 4 months, using pentobarbital sodium (100mg/kg, I.P); blood was collected by cardiac puncture, and plasma levels of glucose and lipids determined [1]. The study was approved by the institution's Animal Ethics and Welfare Committee, and procedures performed according to the UK Animals (Scientific Procedures) Act, 1986. Gene and protein expression in liver samples and stably transfected cell lines were determined by Q-PCR and immunoblotting; radiolabelled precursors were used to measure lipid synthesis and secretion, and lipidation of exogenous apolipoprotein (apo) A-I [2-4].

There was no change in hepatic expression of StarD1 mRNA in obese rats, compared (cf.) with lean rats; low levels of StarD1 protein were difficult to detect by immunoblotting. Hepatic levels of StarD3 mRNA (48%; $p < 0.01$; $n = 4$) decreased in obese male cf. lean male rats. Levels of StarD3 protein decreased in obese male (11.65-fold; $p < 0.01$) and obese female (2.31-fold; $p < 0.001$) rats, cf. lean controls, linking StarD3 with hepatic storage or export of lipids. Overexpression (OE) of StarD1 (10-fold; $p < 0.05$) increased [3H]glycerol (0.25mM) and [14C]oleate (0.7mM) incorporation (2h) into cellular triglycerides by 2.3-fold ($p < 0.05$) and 1.4-fold ($p < 0.05$). StarD3 OE (1.3-fold; $p < 0.05$) increased synthesis of triacyl[3H]glycerol by 2.0-fold ($p < 0.05$), and [14C]acetate (0.5mM; 2h) incorporation into the same pool by 1.8-fold ($p < 0.05$) cf. EV. OE of StarD3, but not StarD1, increased lipidation of apoA-I (10 μ g/ml; 24h) by 1.7-fold ($p < 0.01$) cf. EV. Finally, StarD3 OE significantly ($p < 0.01$) altered expression of genes which impact on hepatic insulin resistance, inducing Ppargc1a (8.6-fold), Cyp2e1 (2.8-fold), Nr1h4 (2.4-fold), G6pc (5.9-fold) and Irs1 (2.6-fold), and repressing Scl2a1 (2.8-fold), Igfbp1 (2.1-fold), Casp3 (2.1-fold) and Serpine 1 (3.8-fold) cf. EV.

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In conclusion, targeting StarD3 may increase circulating levels of HDL and protect the liver against lipotoxicity; loss of hepatic expression of this protein, induced by genetic obesity, may contribute to the pathogenesis of steatosis.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC004

Modulation of antioxidant enzymes and inflammatory cytokines: Possible mechanism of anti-diabetic effect of ginger extracts

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Ginger (*Zingiber officinale*) is a commonly used food spice in many Asian and African countries. Several studies have demonstrated that ginger is endowed with hypoglycaemic properties under normal and diabetic conditions (Alli et al, 2008). Treatment with ginger extract produced a significant reduction in fasting blood glucose, serum lipids and glucose intolerance in diabetic rats (Akhani et al, 2004). Although oxidative stress and inflammation are reportedly involved in the pathophysiology of diabetes mellitus (Perez – Matute et al, 2009), it is not known if ginger's anti-diabetic effect modulates antioxidant enzyme activity and inflammatory cytokines under diabetic condition. This study employed streptozotocin-induced diabetic rats to investigate the impact of aqueous and ethanol extracts of ginger on activities of superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) as well as malonaldehyde (MDA) and tumor necrosis factor-alpha (TNF) level. Diabetic rats were intra-gastrically given aqueous ginger extract (AGE 250 or 500 mg/kg) and ethanol ginger extract (EGE 250 or 500 mg/kg) daily for 42 consecutive days.

Poster Communications

A separate group of diabetic rats given placebo served as positive control. Blood glucose measurement was determined using glucose oxidase method and TNF- α was assayed using ELISA kits. Oxidative analyses of the liver homogenate were carried out using previously described standard methods (Morakinyo et al 2011). Data are expressed as mean \pm S.E.M., compared by ANOVA followed by Tukey Kramer post-hoc test. Both fractions of the ginger extracts employed significantly reduce the blood glucose level of treated rats compared with placebo-treated diabetic rats (110 ± 12.37 mg/dl for AGE 250 and 115 ± 9.72 mg/dl for EGE 250 vs 338 ± 10.27 mg/dl for placebo). The treated diabetic rats which had received AGE and EGE showed significantly ($p < 0.05$) increased SOD, CAT and GSH activities. The extracts however produced a significant decrease in MDA (1.38 ± 0.05 nmol/mg for AGE 250 and 1.39 ± 0.08 nmol/mg for EGE 250 vs 1.76 ± 0.07 nmol/mg for placebo) and TNF- α (6.82 ± 0.23 pg/ml for AGE 250 and 6.94 ± 0.27 pg/ml for EGE 250 vs 8.21 ± 0.32 pg/ml for placebo) level at the end of the experiments. These data therefore suggest that the mechanism of anti-diabetic effect of ginger may in part involve suppression of oxidative stress and inflammation respectively through the enhancement of endogenous antioxidant activities and reduction of inflammatory cytokines.

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PC005

Evidence for a role of GHSr1 constitutive activity in the acute control of food intake in freely feeding mice

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Ghrelin is the only circulating hormone known to stimulate food intake. However, a paucity of good pharmacological tools has prevented a full understanding of the role of ghrelin, and its cognate receptor GHSr1 in the physiological control of feeding. We have identified a series of GHSr1 ligands with varied pharmacology, including full and partial agonists and inverse agonists, with PK properties suitable for in vivo use. We have used these to probe the role of GHSr1 in the normal diurnal control of food intake in mice.

Poster Communications

Freely feeding mice (C57Bl6J Ola/Hsd) were orally dosed with GHSR1 ligands at the beginning of the dark phase, when feeding behaviour was most active. Doses were chosen to achieve unbound plasma and/or brain concentrations above the in vitro IC50/EC50 at the GHSr1 receptor. Food intake was measured at intervals over the following 24-hours.

GHSr1 partial agonists stimulate food intake in the first hours after administration, and this increase in cumulative food intake is maintained throughout the diurnal cycle, with no compensatory underfeeding at later time points (2hr, 197% cf. vehicle-treated control; 6hr, 132%; 24hr, 119% (all $p < 0.05$)). Conversely, a CNS-penetrant ghrelin inverse agonist decreased food intake in the time period shortly after administration, but this decrease was not maintained, and compensatory overfeeding during later phases of the diurnal cycle meant that there was no overall effect on 24-hr food intake (2hr, 63% ($p < 0.05$); 6hr, 80% ($p < 0.005$); 24hr, 94% $p = \text{NS}$). This was not due to drug exposure, since a second administration of compound was unable to extend the period during which food intake was decreased (6-8 hrs, antagonist T0 104% cf. vehicle treated control, antagonist T0, T4 104% (both NS)). Similar data was obtained using a CNS-penetrant inverse agonist identified by Abbott. Both inverse agonists were without effect in GHSr1 knockout mice (e.g. 6hr, inverse agonist, 93% (NS)).

A difference was detected between the efficacy of CNS-penetrant GHSr1 inverse agonists, and those unable to cross the blood-brain barrier. Whereas the effect of CNS-penetrant lasted first 6 hours, that of an inverse agonists confined to the peripheral circulation had effects only in the first 2 hours after administration (0-2hr, 46% ($p < 0.005$) cf. vehicle; 4-6hr, 106% (NS)). Interestingly, a brain-penetrant neutral antagonist compound had no effect on food intake alone but blocked the feeding stimulated by a GHSr1 agonist. These results suggest that, while GHSr1 activation can increase food intake, in this model, the constitutive activity of GHSr1 receptors in the brain may be more important than circulating ghrelin levels for the physiological regulation of food intake.

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PC006

The effects of a high-fat, high-cholesterol (HFHC) diet on uterine contractile protein expression and ex-vivo contractile activity in term pregnant rats

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The prevalence of maternal obesity is increasing and is associated with uterine contractile dysfunction and emergency caesarean section. The mechanisms through which maternal obesity causes uterine dysfunction are currently unknown. A recent pilot study feeding rats a HFHC diet to induce obesity illustrated negative effects upon uterine contractile associated protein (CAP) expression during labour. This study's aim was to determine whether a HFHC diet has adverse effects on ex vivo uterine contractility and uterine CAP expression in term non-labouring (TNL) and term labouring animals (TL). All animal research was approved by the UK Home Office and carried out under ASPA 1986. 40 female Wistar rats were fed either a control chow (CON n=20) or HFHC (n=20) diet for 6 weeks. Rats were then mated, and maintained on their respective diet throughout pregnancy until day 21 TNL (n=10) or day 22 TL (1st pup delivery) (n=10) and euthanized. Uterine tissue was snap frozen for western blot analysis of CAPs Caveolin-1 (CAV1) & Connexin-43 (CX43). Uterine strips were also dissected and mounted within an organ bath to equilibrate prior to 30 minutes baseline recording of spontaneous contractile activity. Statistical tests using SPSS included independent t test for determination of visceral fat levels and CAP expression and One-way ANOVA for the variation in individual spontaneous myometrial contractions all at the $P < 0.05$ level. HFHC fed rats had significantly greater fat mass, $24.19g \pm 2.29$, versus $12.73g \pm 0.99$ for CON, $P < 0.05$). The HFHC diet significantly increased CAV1 expression in TNL tissue compared to CON ($P < 0.03$), however no difference was observed in TL samples. Uterine CAV1 expression significantly decreased ($P = 0.007$) from TNL to TL within HFHC animals, but no significant change was observed in CON animals. CX43 expression in TNL tissue was significantly higher in HFHC versus CON animals ($P < 0.047$), CX43 expression was lower in TL animals but not to statistical significance ($P < 0.078$). Within dietary groups, CX43 expression was significantly up-regulated with TL in CON rats ($P < 0.001$), however, up-regulation of CX43 with TL was absent in HFHC fed rats. Analysis of baseline spontaneous uterine contractile activity identified a lack of synchronous contractions within the HFHC fed animals compared to CON (Fig.1). Variation in the amplitude of uterine contractions in CON animals improved significantly from TNL to TL ($P < 0.012$). In contrast, rats

Poster Communications

fed the HFHC diet displayed asynchronous contractile activity at TNL. There was improvement with TL but asynchronous activity persisted. In conclusion HFHC diet induced obesity negatively affects synchronisation of uterine contractions at term. This asynchronous contractile phenotype may result from adverse effects of obesity on uterine CAP expression.

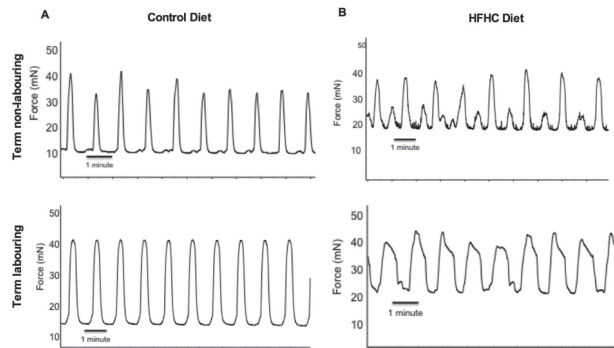


Figure 1 - Representative ex-vivo contractility trace from single term non-labouring (upper traces) and term labouring (lower traces) tissue strips from animals fed a control diet (A) & HFHC diet (B) during 10 minute baseline integral recording.

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PC007

The effects of two consecutive days of exercise on appetite regulatory hormones, appetite perceptions and energy intake in healthy young men

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Appetite regulation is important for maintenance of a healthy body mass. Vigorous exercise suppresses appetite and this is associated with lower levels of the 'hunger hormone' ghrelin and increased levels of the hunger suppressing hormone peptide YY (1-5). These patterns have only been examined in the short term and research has yet to explore the effect of successive days of exercise. This study compared appetite perceptions, energy intake and appetite regulatory hormones on two consecutive days of vigorous intensity exercise.

Fifteen healthy, physically active men (18-24 y) completed two, 2-d trials (exercise and control) in a crossover design. The exercise trial involved a 1-h treadmill run at 70% VO_2max , in a fasted condition, on the morning of each trial day followed

by 6-h of rest. The control trial involved 7-h of rest on each trial day. *Ad libitum* meals provided at 2 and 6-h on each trial day and an *ad libitum* snack bag for the overnight period were used to assess energy intake. Seven blood samples were collected during each trial for the measurement of acylated ghrelin, total peptide YY and insulin by ELISA, and triglycerides and glucose by enzymatic, colorimetric measures. Appetite was assessed at 30-min intervals using visual analogue scales. Data were analysed via two-factor (time x trial) repeated measure ANOVAs and paired sample t-tests using SPSS version 21.0 for Windows. Significance was set at $P < 0.05$, $n = 15$ unless otherwise stated. Values are expressed as mean \pm SD (text) or mean \pm SEM (figure). Energy intake did not differ significantly between the control and exercise trials (6983 ± 957 vs. 6921 ± 796 kcal/trial (31-h)). Area under the curve (AUC) values for hunger and prospective food consumption (PFC) were lower in the exercise vs. the control trial (hunger: 531 ± 148 vs. 610 ± 173 , PFC: 606 ± 140 vs. 687 ± 161) while AUC values for fullness were higher in the exercise trial (772 ± 102 vs. 685 ± 152) (all $P < 0.05$). Acylated ghrelin and total PYY ($n = 14$) did not differ significantly between trials (Figure 1). There was an interaction effect for triglyceride ($P = 0.036$), suggesting a trend for lower concentrations at the end of day two on the exercise trial. AUC glucose concentrations were significantly higher in the exercise vs. the control trial (69 ± 4 vs. 65 ± 6 mmol/L/trial (14-h)), partly due to a glucose spike in the exercise trial after the first buffet meal (3-h) on day two (6 ± 1 vs. 4 ± 1 mmol/L, $P < 0.001$). AUC Insulin concentrations were lower in the exercise vs. the control trial (1414 ± 425 vs. 2065 ± 878 pmol/L/trial (14-h)), with lower concentrations in the exercise trial before the first buffet meal (2-h) on day two (16 ± 6 vs. 29 ± 10 pmol/L, $P < 0.001$).

These findings indicate that there is no compensation for energy intake or alterations in appetite regulatory hormones across two consecutive days of exercise.

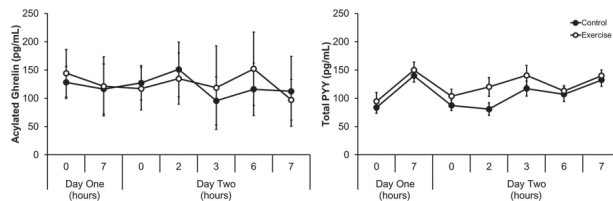


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PC008

Effect of quinine on gastric acid secretion in albino Wistar rats

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Quinine remains an important anti-malarial drug. The 2010 World Health Organisation guidelines recommend a combination of quinine plus doxycycline, tetracycline or clindamycin as second-line treatment for uncomplicated malaria and also for treatment of malaria during first trimester of pregnancy. However, the effects of quinine on many gastrointestinal functions are not known. Therefore this study aims at investigating the effect of therapeutic dose of quinine on gastric acid secretion, which might be important in peptic ulcer patients. Forty albino Wistar rats were randomly divided in 8 groups of 5 animals each. Gastric acid output was measured by the continuous perfusion method in animals anaesthetized with 25g/100ml urethane at a dose of 0.6ml/100g body weight, i.p. After consistent basal gastric output were obtained, each animals in the different groups were treated as follows: group 1 received normal saline (1ml/kg, i.p) administration ; group 2, quinine (10mg/kg, i.m); group 3, carbachol (50µg/kg, i.p); group 4, quinine + carbachol; group 5, atropine (1mg/kg, i.p) + quinine; group 6, histamine (20mg/kg, i.p); group 7, quinine + histamine and group 8, ranitidine (4mg/kg, i.p) + quinine. Values were expresses as mean ± SEM and compared by student t-test. Result showed that peak acid secretion (PAS) in rats treated with normal saline was $1.28 \pm 0.04\text{mEq/L/10min}$. Quinine administration significantly increased the PAS to $2.00 \pm 0.18\text{mEq/L/10min}$ ($p < 0.01$). Carbachol significantly increased PAS to $9.38 \pm 1.33\text{mEq/L/10min}$ ($p < 0.001$). Injection of quinine before carbachol did not significantly affect the PAS as compared with carbachol alone ($p > 0.05$) and atropine administration did not significantly reduce the PAS to quinine ($p > 0.05$). Histamine significantly increased the PAS to $8.08 \pm 0.26\text{mEq/L/10min}$ ($p < 0.001$). Injection of quinine before histamine significantly reduced the PAS to $3.42 \pm 1.12\text{mEq/L/10min}$ ($p < 0.01$) and ranitidine blocked the secretory response of the stomach to quinine. In conclusion, quinine increased gastric acid secretion in rats by stimulating histamine H2 receptors.

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PC009

Elevated maternal plasma DLK1/PREF-1 in pregnancy is conceptus-derived and its abrogation affects maternal metabolic adaptations to pregnancy

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Delta-like homologue 1 (Dlk1, also called Pref-1) is a paternally-expressed imprinted gene that is present in both membrane-bound and soluble forms. *Dlk1* is implicated in postnatal control of metabolic parameters, including the appropriate and timely development of brown adipose tissue (1) and the propensity to develop obesity on a high fat diet (2, 3). In the adult, *Dlk1* expression is low, as is the concentration of soluble DLK1 in the plasma. However, during pregnancy maternal plasma DLK1 levels rise dramatically, with the peak concentration correlating with the number of fetuses carried (4). Thus, we hypothesised that maternal plasma DLK1 during pregnancy may be conceptus-derived. Additionally, overexpression of *Dlk1* from endogenous control elements results in a switch in whole-body metabolism to favour fatty acid utilisation (3). Thus, we hypothesised that the elevation in maternal plasma DLK1 during pregnancy may occur to enable maternal metabolic adaptations to pregnancy.

To test these hypotheses, we utilised genetic mouse models and the imprinting status of DLK1 to selectively abrogate *Dlk1* expression in the mother, conceptus, both or neither. By measuring maternal plasma DLK1 concentrations in these crosses during pregnancy, we demonstrated that the pregnancy-induced elevation in maternal plasma DLK1 does not occur in the absence of *Dlk1* expression in the conceptus. Thus, the conceptus is the source of the additional DLK1.

We analysed maternal metabolic parameters in mothers in the presence and absence of pregnancy-induced elevated maternal plasma DLK1. Moreover, we utilised mothers of varying *Dlk1* expression status, since membrane-bound DLK1 has been demonstrated to be required for the response to soluble DLK1 in other tissue contexts (5). Maternal resource allocation was affected by maternal plasma DLK1 levels, but only in *Dlk1*-null mothers. Abrogation of elevated maternal plasma DLK1 did not affect whole-body weight gain, but had maternal genotype-specific effects on tissue weight gain. Analysis of maternal plasma metabolite levels demonstrated alterations consistent with changes in cholesterol metabolism in *Dlk1*-null

mothers but fatty acid metabolism in *Dlk1*-expressing mothers. Thus, elevated maternal plasma DLK1 during pregnancy affects maternal metabolic parameters in a complex, maternal expression status-dependent manner.

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PC010

SATIN (Satiety Innovation) Project: Consumption of a novel type 3 resistant starch induces distinct changes in gut microbiota of overweight human volunteers

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The dramatic increase in obesity and associated diseases has focused interest on the influence of dietary ingredients in controlling satiety. Dietary resistant starch (RS) undergoes colonic microbial fermentation leading to increased short-chain fatty acid (SCFA) concentrations, which may increase satiety via the SCFA-induced release of gastrointestinal hormones such as glucagon-like peptide-1 and polypeptide YY. This work utilised high-throughput 16S rDNA sequencing to characterise changes occurring in the intestinal microbiota following incorporation of a novel type III RS into the diet. This approach provided a more in-depth and comprehensive analysis than previous qPCR, 454 and microarray-based studies. All subjects (n=22) followed a controlled 44-day crossover dietary plan. Faecal samples were collected during each of the four diets: maintenance (3d), weight-loss (21d), control (10d) and RS type III (C*ActiStar 11700, Cargill) (10d). Extracted microbial 16S rDNA was then sequenced using the Illumina MiSeq platform and analyses were generated using QIIME and Metastats software. To understand how RS affected the faecal microbiota, only samples from the control and RS diets were incorporated into the statistical analyses. The inclusion of RS into the diet significantly changed the faecal

microbiota by altering the abundance of selective groups of diet-responsive taxa ($p < 0.05$, ADONIS, weighted Unifrac). Phylogenetic diversity (PD) did not change following the consumption of RS, indicating that microbial changes were related to abundance rather than incidence (presence/absence) ($p > 0.05$, nonparametric t-test, PD). At the genus level, *Roseburia*, *Ruminococcus* and *Faecalibacterium* were significantly higher following RS consumption, exhibiting average fold increases of 1.5, 1.4 and 1.2 respectively ($p < 0.05$, Metastats). The increased abundance of both *Ruminococcus* and *Roseburia* was largely attributable to changes in *R. bromii* and an unclassified *Roseburia* species ($p < 0.05$, Metastats). *R. bromii* and relatives of *Roseburia* have previously been correlated with increased consumption of an RS type III, however the specific increase in *Faecalibacterium* has not been observed before. Here, using a whole-community approach, we show that three genera which have been associated with either SCFA synthesis and/or starch degradation, are significantly enriched by the consumption of this novel RS. Moreover, these data demonstrate the selective enrichment of a distinct number of “key” species in response to addition of this RS into the diet, possibly due to substrate-specificity at the species level.

The research leading to these results has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 289800

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PC011

Comparative studies on reproductive organ weight and fertility assessment following alcohol and nicotine administration in rats model

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This study seeks to investigate the effect of alcohol and nicotine administration on reproductive organ weight and fertility profile in rats model. Male albino rats (180-200g), were randomly divided into four groups of five rats each as follows. Group 1: control group (distilled water), group 2: alcohol alone (3g/kg bw as 25%v/v), Group 3: nicotine alone (1.0 mg/kg bw), group 4: alcohol (3g/kg bw as 25%v/v) + nicotine (1.0 mg/kg bw). Drug administration via oral administration lasted for 52 days (spermatogenic cycle in rats); before, during and after treatment body weights of all experimental rats were recorded and at the end of the experiment blood was collected via the retro-orbital sinus under ether anaesthesia (for hormonal profile: FSH, LH and Testosterone and antioxidant assays), the animals were sacrificed by cervical dislocation in order to exposed the organ(testis) of choice for histology. Semen analysis was carried out by exposing the testis with the epididymis and the

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epididymis was carefully separated and the caput was removed. The caput was transferred unto a pre-warmed slide and lacerated to release some semen unto the slide surface. Administration of nicotine significantly reduces ($P<0.05$) the weight of the testis and accessory sex organs. A marked significant decrease ($P<0.05$) in sperm profile (motility, count, mature sperm and morphology) was observed in sperm collected from the epididymis of the alcohol, nicotine and alcohol plus nicotine treated animals. However, the present study showed a reduction in reproductive organ weights and lack of offspring after mating affirms the outcome of this study and this suggests that both alcohol and nicotine have antifertility activities with probable site of action as the testis.

Keywords: Alcohol, nicotine, weights, reproduction, infertility, and rats.

The effect on body weight of rats treated with alcohol and nicotine

Group	Initial Body Weight	Final Body Weight	Change in Body weight
Control	194 \pm 4.00	220 \pm 5.48	26 \pm 1.48
Alcohol (3g/kg bw 25%v/v)	191 \pm 4.00	220 \pm 4.47	29 \pm 0.47
Nicotine (1mg/kg bw)	192 \pm 4.90	187 \pm 6.63	5 \pm 1.73*
Alcohol (3g/kg bw 25%v/v)+Nicotine(1mg/kg bw)	184 \pm 4.00	187 \pm 8.60	3 \pm 4.6*

Data are presented as Mean \pm SEM. * $p<0.05$ were considered significant with respect to control

Sperm profile of experimental rats treated with alcohol and nicotine

Group	Sperm count(106/ml)	Mature sperm(%)	Sperm motility(%)	Sperm morphology(%)
Control	96.00 \pm 1.87	86.00 \pm 2.45	90.00 \pm 1.58	12.00 \pm 1.22
Alcohol	38.00 \pm 2.55*	56.00 \pm 2.92*	42.00 \pm 2.55*	45.00 \pm 1.11*
Nicotine	34.00 \pm 1.80*	52.00 \pm 2.55*	40.00 \pm 1.58*	54.00 \pm 1.08*
Alcohol+Nicotine	27.00 \pm 1.00*	50.00 \pm 2.74*	34.00 \pm 1.80*	56.00 \pm 1.02*

Values are presented as Mean \pm SEM, n=5; Significant at * $p<0.05$ when compared to control.

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PC012

A novel role for FTO in adipogenesis

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Genome-wide association studies have revealed that single nucleotide polymorphisms (SNPs) in the first intron of the fat mass and obesity associated (FTO) gene are associated with obesity in humans. Animal studies have since demonstrated that mice overexpressing FTO (FTO-4) have increased body weight compared to wild type (WT) mice. In spite of this, the mechanisms involved in FTO-induced adiposity remain elusive, particularly at the level of the adipocyte. We observed that the adipogenic genes CEBP α and PPAR γ were upregulated (2.85 ± 0.17 - and 2.14 ± 0.16 -fold, respectively) in gonadal adipose tissue of FTO-4 mice compared to WT. We also observed that primary preadipocytes derived from FTO-4 mice exhibit increased triglyceride accumulation and lipid droplet size following adipogenic stimulation compared to that of WT mice. Gene expression analysis of these cells confirmed that differentiated preadipocytes from FTO-4 mice had a significantly higher expression of adipogenic genes, including PPAR γ (56.80 ± 8.07 -fold), FABP4 (51.26 ± 9.71 -fold) and PLIN1 (74.17 ± 6.44 -fold) after 10 days of treatment with an adipogenic cocktail compared to those of WT mice. Similar findings were observed in MEFs derived from FTO-4 mice, in which adipogenic gene expression was significantly elevated compared to WT MEFs after 3 days of differentiation. Conversely, MEFs derived from FTO knockout mice exhibited lower adipogenic gene expression compared to WT MEFs following adipogenic stimulation. Given that FTO-dependent changes in adipogenesis were observed within 3 days of adipogenic stimulation, we hypothesized that FTO was inducing adipogenesis by influencing the early proliferation phase of adipogenesis known as mitotic clonal expansion (MCE). MCE, a prerequisite for adipogenesis, is mainly regulated by Cyclin D1 and D3. We observed that in FTO-4 MEFs, transfection with FTO siRNA lead to a significant downregulation of Cyclin D1 at 24 hours (FTO-4 siCON 1.36 ± 0.12 vs FTO-4 siFTO 0.76 ± 0.04 relative to GAPDH) and Cyclin D3 at 40 hours (FTO-4 siCON 1.35 ± 0.09 vs FTO-4 siFTO 0.62 ± 0.03 relative to GAPDH) post adipogenic induction compared to FTO-4 MEFs transfected with control vector. In conclusion, our results suggest that FTO promotes adipogenesis through regulating MCE.

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PC013

Prevalence of Metabolic Disorders in a University Population and the Effect of a Pre-Prevention Intervention

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Obesity and weight status are implicated in the development of metabolic disorders such as metabolic syndrome (MetS); a cluster of risk factors increasing the occurrence of cardiovascular disease and pre-diabetes (Alberti et al., 2009). The key lifestyle factor of obesity prevalence is elevated waist circumference (WC). The increasing prevalence of such disorders has concurrently been associated to a decreasing age of onset (Hsia et al., 2009). Pre-diabetes and MetS can however, be reversed through lifestyle modification, preventing the onset of chronic disease. It is therefore beneficial to determine the prevalence of metabolic risk in a young diverse student population. The purpose of this study was to determine the prevalence of obesity and metabolic disorders within a University population, and to establish the effectiveness of an intervention to improve weight status and symptoms of metabolic disorders. A cross-sectional study was conducted in a London-based University. A total of 71 participants aged 19-32 years were recruited through random intercept sampling. Stature, mass, body fat %, waist circumference (WC), diastolic and systolic blood pressure (DBP and SBP), fasting blood glucose, cholesterol and triglyceride levels were measured. A non-equivalent control subset (n=32) were recruited to participate in an educational intervention with SMART goal setting to raise awareness of their health, where the control group with no risk factors received no further health advice. Follow-up measures were conducted four weeks later. When BMI was utilised as a measure of obesity, 22.8% of participants were classified as overweight (14.3%) or obese (8.5%). The prevalence of MetS was 1.4%; however no cases of pre-diabetes were identified. Hypertension and elevated cholesterol were the most prevalent risk factors identified in 21.7% and 18.5%, respectively. An elevated WC significantly correlated with elevated SBP, BMI, gender and ethnicity ($P<0.01$). The control and intervention groups differed significantly at baseline for mass, BMI, WC, DBP and bodyfat% ($P<0.05$) and knowledge of metabolic disorders was very low (5.7%). The intervention had no significant effect other than WC ($p<0.05$) which decreased for all participants in the intervention group. Obesity was lower than expected, with 72.8% having a BMI between 18.5 and 25kg/m². Additionally MetS and pre-diabetes prevalence was low. The intervention provided promising results in reducing WC, but further follow-up is essential to determine long-term effectiveness. A more ethnically diverse cohort and the use of impaired glucose tolerance testing may have revealed a higher prevalence of pre-diabetes. However, the unique setting provides an opportunity for effective interventions and early prevention strategies to reduce one's risk to future chronic disease.

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Poster Communications

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PC014

Basal calcium influx in rat white fat adipocytes is mediated via Ca_v1.3 voltage gated calcium channels

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The influx of extracellular Ca²⁺ is implicated in various functions of white fat adipocytes which include glucose utilization, lipolysis and adipokine secretion. Consequently dysregulation of Ca²⁺ influx may be involved in the aetiology of the metabolic syndrome. Since little is known about Ca²⁺ entry pathways in these cells we have addressed this question using Ca²⁺ imaging and molecular biology methods on rat epididymal adipocytes. Data is quoted as medians with 95% confidence intervals. Under basal conditions the intracellular calcium concentration, [Ca²⁺]_i was 130 nM (124-138; n=466). In 42 cells tested, removal of extra Ca²⁺ reversibly decreased this by of 18% (11-27). 20μM verapamil and 20μM nifedipine, blockers of L-type calcium channels, both mimicked the effect of Ca²⁺ removal and prevented Ca²⁺ entry and recovery of basal [Ca²⁺]_i when added just before the re-addition of extracellular Ca²⁺ following its removal. These effects were not shared by 10μM KBR-7943, a blocker of Na⁺-Ca²⁺ exchange. RT-PCR indicated the presence of mRNA for Ca_v3.2, Ca_v1.1, Ca_v1.2 and Ca_v1.3, however Western blots of the membrane fraction only revealed the presence of Ca_v1.3 alpha-subunit. In conclusion our data suggests that a background window Ca²⁺ current through Ca_v1.3 maintains [Ca²⁺]_i in white fat adipocytes; this idea compatible and consistent with their depolarized membrane potential (-30mV, Bentley et al 2014) at which this channel type is expected to be open as well as a previous molecular study that also demonstrates the presence of L-type Ca²⁺ channels in this cell type (Gaur et al 1988).

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PC015

Changes in expression of genes related to development of pulmonary arterial hypertension in lung tissue derived from a rat model of type 2 diabetes

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Pulmonary arterial hypertension (PAH) is a progressive disorder characterised by pulmonary vascular constriction and remodelling which ultimately leads to right heart failure. Recent evidence suggests an association between type II diabetes (T2D) and PAH. Epidemiological data show that patients with T2D are at increased risk of developing PAH (1). Clinically, PAH patients have increased insulin resistance (2), while experimentally, rodent models of insulin resistance develop PAH (3,4). However, the mechanisms associating T2D with the development of PAH are poorly understood. The aim of the current study was to investigate changes in expression of key genes related to the development of PAH in lung tissue derived from a rat model of T2D. Right ventricular hypertrophy (RVH), an indicator of PAH phenotype, was also assessed in T2D rats. **Methods:** T2D was induced in 12 week old male Wistar rats (n=6) by feeding a high fat diet (22%) for 12 weeks, with a single dose of streptozotocin (30mg/kg, i.p.) given on week 4. Control rats (n=5) were fed standard diet and injected with vehicle. T2D was verified by hyperglycaemia and hyperinsulinaemia. Rats were sacrificed at 24 weeks old via sodium pentobarbitone (5mg/100g, i.p.). Whole lung gene expression of bone morphogenetic protein receptor type 2 (BMPR2), tryptophan hydroxylase 1 (Tph1; the rate-limiting enzyme in the synthesis of serotonin), the receptor for advanced glycation end products (RAGE), endothelial nitric oxide synthase (NOS-3) and NADPH oxidase 4 (NOX-4) was analysed by real time PCR and expressed as relative quantity. RVH was expressed as the ratio of the weight of the right ventricle to the weight of the left ventricle plus septum. Data were analysed by unpaired Students t-test and expressed as mean \pm SEM. **Results:** BMPR2 gene expression was downregulated in lungs of T2D rats (control: 1.0 ± 0.31 vs T2D: 0.02 ± 0.09 , $p \leq 0.001$). Additionally a reduced expression of RAGE (control: 1.0 ± 0.21 vs T2D: 0.48 ± 0.20 , $p \leq 0.05$) and NOX-4 (control: 1.0 ± 0.21 vs T2D: 0.14 ± 0.16 , $p \leq 0.05$) was observed in the lungs of T2D rats. No significant alteration in Tph1 (control: 1.0 ± 0.36 vs T2D: 0.43 ± 0.48) or eNOS (control: 1.0 ± 0.38 vs T2D: 1.41 ± 0.64) expression was found. No significant alteration in RVH was observed between control rats (0.232 ± 0.024) and T2D rats (0.246 ± 0.010). **Conclusions:** This study has shown reduced gene expression of BMPR2, RAGE and NOX-4 in the lungs of T2D rats. A reduction in BMPR2 gene and protein expression has been shown in PAH patients and in animal models of disease (5), therefore this may pre-dispose T2D rats to development of PAH. Despite these changes in gene expression, T2D rats did not show RVH. Ascer-

Poster Communications

taining whether pulmonary vascular remodelling occurred in T2D rats is important and currently ongoing.

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PC016

Curcumin (a polyphenolic extract of Turmeric) alters oxidative stress and acrosomal reaction in rat spermatozoa

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Curcumin (CUM), a food additive with international numbering code E100, sold as an over-the-counter supplement worldwide has been shown to exhibit therapeutic potential against some chronic illnesses in which inflammation and free radicals are known to play crucial roles such as Parkinson's disease, cancer, diabetes, testicular damage etc (Aggarwal and Harikumar 2009). However, the effect of Curcumin on normal reproductive tissues has not been sufficiently studied. Therefore, the effect of Curcumin on oxidative balance and acrosomal reaction in rat spermatozoa was studied. Curcumin was extracted from turmeric rhizomes (voucher number LUH 5695) by the method described by Liu *et al* 2008. A concentration of 48mg/ml CUM was prepared for use in the study. Twenty adult male rats were randomly divided into four equal groups. The control group received distilled water; while other groups received 50mg/kg CUM, 100mg/kg of CUM and 150 mg/kg CUM intra-peritoneally (0.2 -0.6mls). The animals were all treated with CUM once daily for fourteen days after which they were sacrificed by cervical dislocation. For sperm collection the testicles and the epididymis were exposed through a lower abdominal incision. The right and left caputs of epididymis were quickly excised from the body of the testes cut longitudinally with a pair of fine-pointed scissors and compressed with forceps to release the sperm cells. Sperm cells from the caput epididymis were finely minced with normal saline in a Petri dish to liquefy and provide migration of all spermatozoa from the epididymal tissue to the fluid (Morakinyo *et al* 2010). Lipid peroxidation and antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), reduced glutathione and acrosomal reaction were determined in the sperm. Calcium ion concentration was also determined in the semen using colometric method as described by Bennett *et al.* (1973). All the concentration of CUM (50mg/kg, 100mg/kg and 150mg/kg) significantly increased ($P < 0.05$)

antioxidant activities of CAT (108 ± 6.76 , 156 ± 18.2 and 109 ± 7.83 respectively compared with the control (27.2 ± 0.38) and SOD (11.5 ± 1.95 , 30.6 ± 4.31 and 19.3 ± 3.99 respectively compared with the control 5.27 ± 0.35) in the epididymal sperm. The percentage value for acrosomal reaction was also significantly increased ($P < 0.05$) in 100mg/kg ($61.25 \pm 1.25\%$) and 150 mg/kg ($72.50 \pm 1.44\%$) CUM treated rat when compared with the control ($43.00 \pm 0.58\%$). Lipid peroxidation was also significantly increased in all CUM treated rats. The three concentrations of CUM produced an insignificant decrease in calcium concentration in the semen. This study showed that curcumin increase lipid peroxidation and also increases antioxidant enzymes. The activities of the antioxidant might have been more than the peroxidation thus the observed enhancement of acrosomal reaction.

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PC017

Obesity in pregnancy courses with endothelial dysfunction and worsens adenosine transport in human umbilical vein endothelium from gestational diabetes

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Obesity during pregnancy (OP) is a condition where mothers with normal body mass index (BMI) develop supraphysiological weight gain during pregnancy ending with obesity ($BMI \geq 30$) (1). Increased risk of gestational diabetes (GD) is associated with OP (~2.6 fold). GD is a condition associated with metabolic alterations of the placental endothelium, leading to altered vascular reactivity (2). Even when obesity is correlated with high cardiovascular risk, and GD associates with reduced equilibrative nucleoside transporter 1 (hENT1)-mediated adenosine transport (3), nothing is known regarding the potential effect of OP on the human fetoplacental

endothelial function. We evaluated whether OP associates with altered nitric oxide (NO) synthesis and whether this pathological condition worsens the GD-reduced hENT1-adenosine transport in human umbilical vein endothelial cells (HUVEC). Umbilical vein reactivity in the absence/presence of 10 μ M L-NAME in response to increasing insulin concentrations (10-10-10-6 M) were measured in umbilical vein rings from normal and OP groups in a wire myograph. L-[3H]Citrulline formation (100 μ M L-arginine, 2.7 μ Ci/ml, 37C, 30 min), adenosine transport (31-250 μ M Adenosine, 3 μ Ci/ml, 25C, 20 sec) in the absence/presence of 1 or 10 μ M nitrobenzylthioinosine, hENT1 and endothelial NO synthase (eNOS) protein and mRNA expression and eNOS phosphorylation, were measured in primary cultures of HUVEC from normal (N) or GD pregnancies coursing without (N or GD groups) or with (OP or GD+OP groups) OP. Insulin-mediated vasodilation was almost absent in vein rings from OP. L-Citrulline formation was only decreased in OP (~96%) in HUVEC. Moreover, eNOS expression is reduced in OP (~42 and 80% for protein abundance and mRNA expression, respectively), but only protein abundance is lower in GD (~38%) compared to N. The mRNA expression was also reduced (~90%) in GD+OP. Higher inhibitory (p-Thr495) phosphorylation of eNOS was found in OP compared with the other groups. Adenosine transport via hENT1 is reduced in OP, GD and GD+OP compared with N group (V_{max}/K_m : $0.25 \pm 0.04^*$ N; 0.11 ± 0.01 OP; 0.15 ± 0.03 GD; 0.005 ± 0.02 GD+OP, 1way ANOVA; * $p < 0.05$). However hENT1 protein abundance and mRNA expression are increased in OP (~1.7 and ~3-fold, respectively), but reduced in GD (~60%) and GD+OP (~50%) compared with N group. In conclusion, OP is a pathological condition that results in lower NO synthesis; meanwhile in women with OP and GD inhibition of adenosine transport is likely due to reduced hENT1 transport activity via mechanisms that is independent of NO synthesis.

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PC018

Proglucagon-derived peptides and exendin-4 do not increase acute exocrine secretion in vivo and in vitro in rat

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Glucagon-like peptide 1 (GLP-1) receptor agonists are currently widely used in the treatment of Type 2 Diabetes Mellitus, and are being investigated as a potential anti-obesity therapy thereby increasing the target group of patients. However, safety concerns have been raised from reports suggesting a link between GLP-1 based treatments, high pancreatic ductal replication and increased amylase secretion from acinar cells, potentially increasing the risk of pancreatitis. This has triggered a number of in vivo chronic studies with conflicting results. However little is known about the acute effect of GLP-1 on functions of the exocrine pancreas. Amylase secretion in vivo and in vitro was assessed in the four main peptides intended to be used as obesity therapeutics. . To evaluate the acute exocrine effect in vivo, male Wistar rats (350-500g) were anaesthetised using isoflurane (1.5-2%), and an intravenous bolus injection of 30 nmol GLP-1 (7-36), glucagon, oxyntomodulin or exendin-4, alone and in combination with cholecystokinin (CCK), was administered through the femoral vein (n= 3-5). Plasma levels of amylase were determined from right jugular vein sampling performed at 50 mins post injection. Results were compared to baseline pre-treatment plasma and presented as means \pm SEM by ANOVA. Rat pancreatic acinar cells (AR42J) were treated with ascending concentrations of the peptides and CCK, and medium was assayed to evaluate amylase secretion. Plasma amylase concentration did not increase post peptide injection in vivo, compared to vehicle and CCK. Of note, when oxyntomodulin was co administered with CCK, it suppressed amylase secretion by 4 μ mol/ml \pm 0.3, compared to CCK alone. Nonetheless, in vitro amylase secretion post oxyntomodulin/ CCK cellular treatment was not significantly suppressed, when compared to CCK. These results suggest that GLP-1, glucagon and exendin-4 do not have an acute effect on exocrine pancreatic function, while oxyntomodulin appears to suppress digestive enzyme secretion, yet not directly through down regulation of amylase release. Thus, it could be the safest choice for an obesity treatment, with regard to pancreatitis.

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PC019

Improvements in diet quality in the UPBEAT study: A lifestyle intervention to improve outcomes in obese pregnancy

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Obesity during pregnancy increases the risk of serious morbidities and mortality for both the mother and child. Improving dietary intake and physical activity in obese pregnant women may reduce these health burdens. The UK Pregnancies Better Eating and Activity Trial (UPBEAT) is a lifestyle intervention principally aimed at reducing the incidence of gestational diabetes and macrosomia in obese pregnant women and their offspring. The aim of the present study was to assess overall diet quality, using the Alternative Health Eating Index for Pregnancy (AHEI-P), in participants of UPBEAT and to determine the effects of the intervention on diet quality. In brief, UPBEAT is a multicentre randomized controlled trial comparing the effects of a lifestyle intervention, aimed at reducing glycaemic load and saturated fat and increasing physical activity, with usual care in obese pregnant women. Dietary intake was assessed by structured 24-h recalls in 183 women participating in the UPBEAT pilot phase, and analysed using WISP dietary analysis software. The AHEI-P index of diet quality was applied to the dietary data at baseline (15+0-18+6 weeks gestation) and following the intervention (27+0-28+6 weeks gestation), and changes in diet quality assessed. AHEI-P is a 90-point scale based on intakes of fruit, vegetables, white and red meat, fibre, trans fats, polyunsaturated and saturated fats, folate, calcium and iron. Mann-Witney test of difference was used to compare intervention and control groups at baseline. Analysis of covariance was used to compare post intervention AHEI-P scores between groups, adjusting for baseline values. Relationships between diet quality and sociodemographic factors were explored using Pearson correlation and chi-squared tests.

Mean AHEI-P scores were low prior to the UPBEAT intervention (intervention group 39.9 ± 8.4 , control group 43.1 ± 9.5). Following the intervention, there was a significant difference in AHEI-P scores between the groups (intervention group 43.6 ± 8.5 , control group 43.5 ± 9.3 , $p = 0.004$). Significant group differences were observed for the vegetable ($p = 0.001$), white to red meat ratio ($p = 0.005$), fibre ($p = 0.045$), polyunsaturated to saturated fat ratio ($p = 0.014$), trans fat ($p < 0.001$), calcium ($p = 0.017$), iron ($p = 0.014$) and folate ($p = 0.013$), components. Baseline AHEI-P scores were positively correlated with age ($r = 0.174$, $p = 0.019$), and years in full-time education ($r = 0.207$, $p = 0.005$). There was a negative correlation between baseline AHEI-P scores and body mass index ($r = -0.204$, $p = 0.006$).

In conclusion, we have found poor diet quality in a cohort of obese pregnant women. However, following the UPBEAT lifestyle intervention there were significant improvements in diet quality, which may be effective in improving pregnancy outcomes for this high risk group.

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PC020

Holding mice at an environmental photic cycle that matches their endogenous circadian rhythm period length prevents diet induced obesity

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Circadian regulation is responsible for the maintenance of endogenous physiological rhythms, and their temporal organization and entrainment to the environmental (24 h) light-dark photic cycle. Mechanisms underlying circadian regulation and energy homeostasis are interconnected: disruption of the former (i.e., circadian disruption) is accompanied by higher susceptibility to diet-induced obesity (DIO) due to feeding on high-fat diet (HFD). Endogenous circadian rhythms show a period length (τ) that usually deviates from 24 h. Recent descriptive studies have shown that deviation of τ from 24 h is in correlation with mice inter-strain susceptibility to DIO. These studies support our hypothesis that a lack of resonance between τ and environmental period length exacts a metabolic cost increasing susceptibility to DIO. However, this hypothesis has never tested in a controlled setup. Our goal was to conduct controlled animal experiments designed to directly examine whether the deviation of τ from the environmental photic cycle is associated with higher susceptibility to the DIO. This hypothesis was tested by comparing newborn female FVB/N mice rate of obesity, while feeding on a HFD (60% of calories from fat) vs. a low-fat diet (LFD, 10% of calories from fat), under two photic regimes: a 23.7 h photic cycle (half-dark half-light hours) that matches their τ , and a 24 h photic cycle (half-dark half-light hours). Our results show that when raised under a τ -mismatching regular 24 h photic cycle, these female FVB/N mice develop DIO, defined by the difference in body weight and fat % (measured by NMR) under a HFD vs. a LFD (Fig. 1A). However, when raised under their τ -matching 23.7 h photic cycle, DIO was prevented (Fig. 1B). In all, mice fed a HFD had a significantly lower body weight and fat % under a τ -matching 23.7 h photic cycle vs. a τ -mismatching regular 24 h photic cycle. As far as we know, this is the first controlled experiment showing that having a non-24 h period length of the endogenous circadian rhythm enhances the propensity to DIO under the regular 24 h photic cycle. Our results offer a novel risk factor to obesity that justify the identification of biomarkers related to τ for clinical use as a diagnostic risk factor for obesity; and an experimental paradigm for the development of novel, clock-related pharma-

ceutical prophylactic/therapeutic interventions to reduce the gap between internal and external circadian rhythms, and hence reduce the prevalence of obesity.

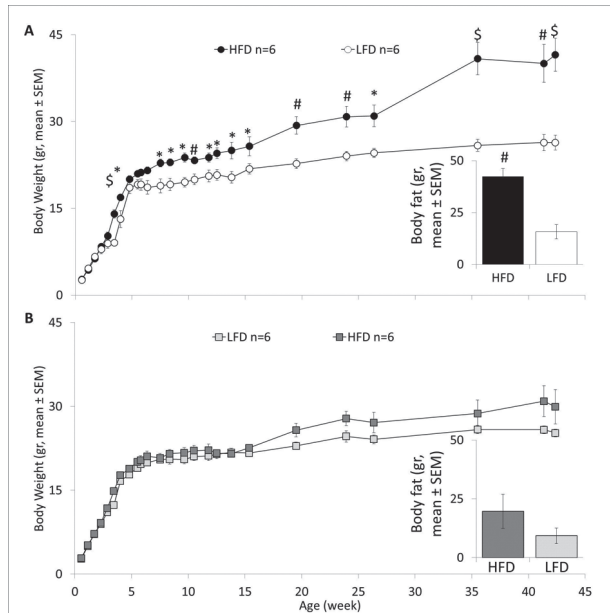


Figure 1: The effect of feeding on a high-fat diet vs. a low-fat diet on body weight and body fat percentage of newborn FVB/N female mice held under the regular 24 h (half-dark half-light) photic cycle (A) or under their tau-matching 23.7 h (half-dark half-light) photic cycle (B). Each time point represents the mean (\pm SEM) of 6 FVB/N female mice. Insert, mean body fat percentage \pm SEM measured by NMR at experimental ending (n=6 in each group). HFD, high-fat diet. LFD, low-fat diet. *, $P < 0.05$; #, $P < 0.01$; \$, $P < 0.001$ between diets by Student's T-test.

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PC021

Association of markers of obesity, oxidative stress and sperm function in in-utero exposure to omega-9 in rats

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Obesity is becoming an epidemic and about 2 billion adults are classified as obese. Male obesity in reproductive-age men has also nearly tripled in the last 30 years which coincides with an increase in male infertility worldwide (Palmer et al.2012).

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Nutrition, the most influential environmental factor during fetal development is accepted as the primary factor that causes programming effect (Amarasekera et al., 2013). Evidences abound to support that early life nutritional patterns are linked to an increased incidence of obesity. A lot of studies have thus related high sugar and high fat diets to obesity. Our study intends to focus on the effect of in-utero exposure to omega -9 (oleic acid) and if it has an influence on anthropometrical index in relation to sperm functions and oxidative stress levels in male pups. Pregnant rats (190-220g, n=20) were divided into first (D7) and second (D14) trimesters and administered oleic acid at 1000mg/kg, a no-observed-adverse effect level (NOAEL) according to the OECD guidelines (OECD, 2003) by oral gavage. The control group was administered olive oil the vehicle for the oleic acid administration. Rats were allowed to deliver naturally while pups were monitored from birth up to 60 days. Food consumption, energy intake, body weight, body length, body mass index (BMI), Lee index and sperm functions were measured. After post-mortam, the testis from each rat was carefully collected and weighed. They were homogenized and used for oxidative analysis. Superoxide dismutase (SOD) activity was determined (epinephrine in 0.005 N HCl). Lipid peroxidation was also analyzed by the formation of malondialdehyde (MDA) Values are mean \pm SEM, compared with ANOVA to show significant differences among groups, Tukey's post hoc test was used to determine specific pairs of groups that were statistically different (GraphPad Software, USA). Body weight increased with increasing age and by day 60, food consumption (control vs D14; 3.78 ± 0.09 vs 3.0 ± 0.03) energy intake (control vs D14; 47.4 ± 1.13 vs 37.6 ± 0.37) and body length (control vs D14; 13.3 ± 0.2 vs 12.0 ± 0.0) were lower in the D14 group when compared with the control while the BMI (control vs D14; 0.44 ± 0.01 vs 0.48 ± 0.00) and Lee index (control vs D14; 0.32 ± 0.01 vs 0.34 ± 0.01) were significantly higher in the D14 group when compared with the control. There was also a significant reduction ($P < 0.001$) in sperm motility, morphology and viability of the D7 and D14 groups when compared with the control. The SOD (control vs D14; 7.2 ± 0.45 vs 2.4 ± 0.05) and GPx (control vs D14; 1.84 ± 0.04 vs 0.6 ± 0.0) levels were significantly reduced in the D14 groups when compared to the control. The MDA (1.0 ± 0.09 vs 5.10 ± 0.08) level was significantly higher in the D14 group when compared to the control. Alterations in BMI were associated with oxidative stress levels and sperm functions in the rats.

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PC022

Developmental abnormalities in the hypothalamus of *Gnasxl* deficient mice underlie elevated energy expenditure, sympathetic activity and cardiovascular physiology

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Gnasxl, an alternative transcript of the *Gnas* locus, encodes an NH₂-terminal variant (XL α s) of the trimeric G-protein subunit G α s. Both proteins stimulate cAMP signalling. *Gnasxl* transcription is epigenetically regulated by genomic imprinting. Mice deficient for *Gnasxl* show a postnatal phenotype of undernutrition and growth retardation, while adults are healthy, but remain lean and hypermetabolic (1, 2). Increased expression of lipolytic genes in adipose tissues, as well as urinary catecholamine levels, indicate an increased sympathetic nervous system activity (2), which also causes higher blood pressure and heart rate in *Gnasxl* knock-outs (KO) (3). *Gnasxl* (XL α s) is expressed in regions of the postnatal and adult brain that regulate energy homeostasis and sympathetic outflow, including hypothalamic nuclei (Arc, PVH, DMH, LH), the suprachiasmatic nucleus and the preoptic area (4). In the medulla the protein is found in neurons of the raphe pallidus, NTS and ventrolateral medulla.

To investigate changes in gene expression, we undertook a RNAseq analysis (Illumina) of total RNA from adult hypothalami. RNAseq data were verified by qRT-PCR and immunohistochemistry (IHC). Developmental histological analyses were performed at postnatal stages P1, P5, P10 and P15.

We identified *Glial Fibrillary Acidic Protein* (*GFAP*) as a transcript that is downregulated in *Gnasxl* KO hypothalami (RNA seq: 2.1 fold down, $p < 0.0001$), qRT-PCR: 2.2 fold down, $p < 0.01$, t-test, $n = 6$ WT, 6 KO). IHC of adult hypothalamus sections revealed a 43% reduction of GFAP-positive cells (201 ± 16 vs 353 ± 22 average cell number/section \pm S.E.M, KO vs WT, $p < 0.0001$ t-test, $n = 23$ sections from 4 WT and 4 KO). This reduction in GFAP-positive cells included astrocytes as well as subpopulations of ependymal tanycytes. Since *Gnasxl* is not expressed in glial cells, we investigated whether these changes might develop as a consequence of the early postnatal failure-to-thrive phenotype of *Gnasxl* KO mice. While glial cell counts of P1 and P5 samples showed no difference, a reduction became apparent on P10 (100 ± 4 vs 125 ± 5 average cell number/section \pm S.E.M, KO vs WT, $p < 0.001$ t-test, $n = 21$ sections from 2 WT and 2 KO) and P15 (86 ± 5 vs 109 ± 5 , KO vs WT, $p = 0.001$ t-test, $n = 37$ sections from 2 WT and 2 KO). Additionally to changes in *GFAP* expression, we found a 2-fold increase in expression of the signalling form of the Leptin receptor (*Lepr-b*) by qRT-PCR ($p < 0.05$ t-test, $n = 6$ WT, 6 KO).

Our findings of reduced *GFAP* expression and glial cell numbers hint at an abnormal postnatal hypothalamic development in lean *Gnasxl* KO mice. The data contrast with descriptions in obese mice of gliosis and elevated GFAP levels. Further work

is aimed at clarifying whether lack of XL α s leads to developmental defects in the organisation of hypothalamic neural/glial circuits.

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PC023

Weight losing and antihyperlipidemic activities of the alkaloid fraction of *Hunteria umbellata* seed in experimental hyperlipidemia

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Water infusion of *Hunteria umbellata* (K. Schum) Hallier f. seed is highly valued in African folk medicine for the local management of diabetes, hyperlipidemia and obesity. Earlier studies have reported the antihyperglycemic, anti-obesity and antihyperlipidemic activities of the aqueous seed extract of this plant in different experimental models. The present study, however, investigates the weight losing and antihyperlipidemic potentials of 25 and 50 mg/kg of the crude alkaloid fraction (HUAf) in normal and triton-induced hyperlipidemic rats, *Hunteria umbellata* seed being known to be a rich source of alkaloids. In addition, the possible weight losing and antihyperlipidemic activities of this alkaloid fraction were investigated. Adult male Wistar rats (weight range: 120-150 g) were randomly divided into 4 and 5 treatment groups in the normal and triton-induced hyperlipidemic models, respectively, and were treated for 14 days before they were humanely sacrificed under inhaled diethyl ether anesthesia. Five (5) ml of whole blood was obtained by cardiac puncture from each treated rat from which serum for lipids assay was subsequently separated from. Results showed that repeated daily oral treatments of normal rats with 25 and 50 mg/kg of HUAf dissolved in distilled water in dissolved in 10% Tween 20 (9:1) for 14 days resulted in significant ($p < 0.05$ and $p < 0.001$) and dose-dependent weight loss, decreases in the serum triglyceride (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDL-c) while significantly

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($p < 0.001$) increased the serum levels of high density lipoprotein-cholesterol (HDL-c) fraction. Similarly, oral pretreatments with 25 and 50 mg/kg of HUAf for 14 days before hyperlipidemia induction with intraperitoneal injection with triton WR 1339 significantly ($p < 0.01$, $p < 0.001$) and dose-dependently attenuated increases in the average body weights, serum levels of TG, TC, LDL-c while also significantly ($p < 0.01$, $p < 0.001$) and dose dependently attenuated significant ($p < 0.001$) decrease in the serum HDL-c levels when compared to the untreated control values. However, the results obtained for 50 mg/kg of HUAf in both normal and triton WR 3339-induced hyperlipidemic rats were comparable to that recorded for 20 mg/kg of simvastatin. In conclusion, the results of this study show that repeated oral treatments with 25 and 50 mg/kg/day of HUAf have weight losing and antihyperlipidemic effects which were mediated via enhanced lipids biliary excretion.

Effect of repeated daily oral treatment with 25-50 mg/kg/day of HUAf on the lipid profile in triton WR-1339 induced hyperlipidemic rats

Groups	TG (mg/dl)	TC (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
I	95.17 \pm 1.94	85.83 \pm 1.97	47.17 \pm 1.17	21.50 \pm 0.89	17.17 \pm 0.75
II	161.50 \pm 1.92 ^c	129.70 \pm 1.43 ^c	25.00 \pm 1.69 ^f	70.83 \pm 4.18 ^c	33.83 \pm 1.76 ^c
III	116.00 \pm 5.03 ^f	100.80 \pm 4.05 ^f	59.17 \pm 3.89 ^{c+}	18.83 \pm 1.60 ^f	18.83 \pm 1.60 ^f
IV	134.30 \pm 6.24 ^c	115.00 \pm 4.03 ^c	60.33 \pm 1.23 ^{c+}	35.50 \pm 2.19 ^f	19.17 \pm 1.68 ^f
V	125.8 \pm 2.14 ^f	105.80 \pm 3.79 ^f	69.17 \pm 3.05 ^{c+}	23.67 \pm 3.63 ^f	13.33 \pm 2.46 ^f

^c and ^f represent significant increase and decrease at $p < 0.001$, respectively, when compared to Group I values while ^e and ^f represents significant decreases at $p < 0.01$ and $p < 0.001$, respectively, when compared to Group II values. ^{c+} represents a significant increase at $p < 0.001$ when compared to Group II values

I = 10 ml/kg of 10% Tween 20-distilled water (p.o.) + 1 ml/kg distilled water (i.p.)

II = 10 ml/kg of 10% Tween 20-distilled water (p.o.) + 200 mg/kg triton WR-1339 (i.p.)

III = 10 mg/kg simvastatin in 10% Tween 20-distilled water (p.o.) + 200 mg/kg triton WR-1339 (i.p.)

IV = 25 mg/kg HU_{Af} in 10% Tween 20-distilled water (p.o.) + 200 mg/kg triton WR-1339 (i.p.)

V = 50 mg/kg/day of HU_{Af} in 10% Tween 20-distilled water (p.o.) + 200 mg/kg triton WR-1339 (i.p.)

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PC024

Exposure to a high fructose diet during pregnancy remodels the intestinal and body composition in the near-term pregnant offspring

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Background and aims: Excess caloric load or a proinflammatory diet may contribute to gut barrier dysfunction, leading to increased nutrient uptake and components of the microbiota passing into the systemic circulation. These effects have been linked to the development of obesity and the metabolic syndrome^{1,2}. Maternal diet and diabetes in pregnancy have been shown to adversely affect many aspects of offspring development. This study examined the effect of a high fructose diet during gestation on gut permeability in female, pregnant offspring.

Methods: Female Wistar rats were placed on either 10% fructose (f) or distilled water (c) at 8 weeks of age and were mated at 10 weeks, with diet continuing through gestation. The offspring were then maintained on the same diet as their dams (c, n=10 and f, n=10) starting from 1 week post-weaning. The offspring were then mated at 10 weeks of age, with tissue collected at gestational day 20 (GD20). Their body composition was determined by MRI directly prior to euthanasia. Ileum and jejunum sections were taken from the small intestine and snap-frozen followed by total RNA extraction for quantitative PCR. Four epithelial tight junction genes were used as markers of intestinal permeability: claudin-3 (CLDN-3), occludin (OCLN), junctional adhesion molecule A (JAMA) and zonulin-1 (ZO-1). Gene expression was measured relative to GAPDH and RPLP0. Groups were compared using t-test or Mann-Whitney U test as appropriate. All values are mean±SEM.

Results: Birth weight was significantly reduced in the f group (c=4.23g±0.5 vs f=3.94g±0.8, p<0.05). Adult weights were unaffected but lean mass reduced and fat mass raised (% lean c=75.8±0.3 vs f=71.4±1.0, p<0.05 and % fat c=11.4±0.5 vs f=16.8±1.3, p<0.01). Gut length was also reduced in the f group (c=128.5cm±1.8 vs f=123.8cm±0.4, p<0.05) and was accompanied with reduced gene expression (p<0.05) for JAMA (0.62±0.06), OCLN (0.44±0.08) and ZO-1 (0.39±0.05) in the jejunum, but not the ileum.

Conclusion: A high-fructose diet during pregnancy may adversely affect gut development in the offspring via a decrease in the expression of genes coding for a number of epithelial tight junction proteins. This response appears to be confined to the jejunum and may contribute to an increase in energy uptake across the gut, thereby leading to an increase in fat mass at the expense of skeletal muscle. Lam, YY *et al.* (2011) "Role of the Gut in Visceral Fat Inflammation and Metabolic Disorders." *Obesity* 19, no. 11: 2113–20.

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PC025

Obesity is associated with altered muscle protein synthetic and breakdown responses to increased nutrient delivery in older adult humans, but not reduced muscle mass or contractile function

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The incidence of obesity is increasing among older adults (Fakhouri et al., 2012), yet despite the central need to maintain muscle mass and function to preserve quality of life, the effect of obesity on muscle protein turnover in this population remains unknown. To investigate this, 11 obese (age: 66.4 ± 1.8; BMI: 31.9 ± 1.1) and 15 lean [age: 66.7 ± 1.1; BMI: 23.4 ± 0.3] male subjects underwent assessment of muscle protein synthesis (MPS) and leg protein breakdown (LPB) under hypoinsulinemic (5 mU.l⁻¹; post-absorptive) and hyperinsulinemic (40 mU.l⁻¹; post-prandial) euglycaemic (4.5 mmol.l⁻¹) clamp conditions, the latter administered in conjunction with mixed-amino acids (10 g.h⁻¹). Leg fat and lean mass were assessed by dual-energy x-ray absorptiometry, along with quadriceps isometric strength of the dominant leg. Statistical differences in anthropometric measures and leg glucose disposal rate were determined using unpaired Student's t-test. Differences in MPS and LPB were determined by 2-way ANOVA; when a significant effect was observed a Student's t-test with Šidák correction was performed to locate differences. Significance was accepted when P < 0.05 and values reported as mean ± SEM. Obesity was associated with a significant increase in leg fat mass (lean: 5.7 ± 0.5 kg; obese: 9.4 ± 0.6 kg; P < 0.001), whilst leg lean mass (lean: 9.6 ± 0.4 kg; obese: 10.1 ± 0.4 kg) and quadriceps strength (lean: 34.3 ± 2.5 kg; obese: 33.9 ± 3.0 kg) were unaffected. Under post-absorptive conditions, MPS and LPB were not different between groups. Insulin and amino acid administration significantly increased muscle fractional synthetic rate of myofibrillar proteins in lean (0.047 ± 0.004 to 0.099 ± 0.011 %·h⁻¹; P < 0.001) but not obese subjects (0.045 ± 0.004 to 0.062 ± 0.006 %·h⁻¹; n.s.), but this blunting of MPS was offset by a parallel decline in LPB in the obese (48.5 ± 9.5 to 29.9 ± 5.5 nmol.min⁻¹.100g leg mass⁻¹; P < 0.05) but not lean (49.8 ± 8.5 to 44.7 ± 7.2 nmol.min⁻¹.100g leg mass⁻¹; n.s.) individuals. Obesity also resulted in a 63% reduction in leg glucose disposal rate under steady state conditions in the post-prandial state (P < 0.001). Thus, obesity in the older adult is associated with a decline in muscle metabolic quality, evidenced by reduced glucose disposal and

blunting of the protein synthetic response to amino acids, but this does not lead to a decline in muscle mass in part due to a reciprocal decrease in LPB rates.

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PC026

Maternal dietary supplementation of fatty acids and its effects on milk composition and adipose tissue development in the offspring

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Background and Aims: Brown adipose tissue (BAT) is essential in enabling the newborn to effectively adapt to cold exposure of the extra-uterine environment (1). In sheep, during postnatal development BAT is rapidly replaced by white adipose tissue, an adaptation determined in part by the maternal metabolic and endocrine environment (2). One dietary factor that may influence this process is the milk content of conjugated linoleic acid (CLA) which, in vitro, has been shown to promote the abundance of the BAT specific uncoupling protein (UCP) 1. In most mammals, the cis-9, trans-11 isomer is the most abundant CLA isomer in milk (3). The aim of this study was, therefore, to determine whether maternal dietary supplementation to increase milk CLA, in particular the cis-9, trans-11 isomer, would promote the retention of BAT during postnatal development.

Methods: From the first day of lactation, sheep were fed daily either a standard diet (n=8) of concentrate (1.5 kg/day) with hay (1.5/kg day) or the same diet plus 3% sunflower oil (SO; n=7). Each mother raised 2 lambs. Milk samples were collected from each mother at 7 and 28 days of lactation, immediately before one lamb was blood sampled, euthanased with an intravenous injection (Pentobarbital Sodium, 200mg/kg body weight) and tissue sampled. Samples of perirenal adipose tissue were weighed and stored at -80°C, then analysed for gene expression for UCP1. This work was carried out under UK Home Office approval. Values are means \pm S.E.M. compared by an unpaired T test.

Results: Supplementation of the maternal diet with SO resulted in an increase of the total CLA at 28 days (Control, n=8, 7.247 \pm 0.60; SO, n=7, 13.54 \pm 0.54 mg/g fat, p<0.0001). The most abundant isomer, cis-9, trans-11 was also higher in the SO group (Control, n=8, 6.3 \pm 0.53; SO, n=7, 11.64 \pm 0.51 mg/g fat, p<0.0001). This was accompanied with a greater abundance of perirenal adipose tissue in females (Control 10.23 \pm 2.06; SO 18.04 \pm 1.03 g/kg bodyweight, n=5 per group, p=0.005). There were also gender differences in the SO group, with males possessing less

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perirenal adipose tissue (Females, $n=5$, 18.04 ± 1.03 ; males, $n=4$, 7.42 ± 0.74 g/kg body weight, $p < 0.0001$). The greater fat mass in female offspring fed SO was also accompanied by an increased mRNA expression of UCP1 (Control 0.36 ± 0.02 ; SO 0.47 ± 0.03 arbitrary units, $n=5$ per group, $p=0.01$).

Conclusions: The addition of SO to the maternal diet increases the cis-9, trans-11 CLA content of the milk and promotes fat deposition in the female offspring. Future analysis will determine whether this is depot specific and the extent to which amount of UCP1 is also raised.

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PC027

The impact of high energy intake during early postnatal life on gene regulators for thermogenesis, adipogenesis and metabolism in the sternal and subcutaneous adipose tissue depots of sheep

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Background and aims - The early life nutritional environment affects adipose tissue growth and susceptibility to later obesity and related adverse health (1). Using sheep as a model, we examined the effects of excess energy intake in early post-natal life on the growth and development of sternal and subcutaneous adipose tissue. We hypothesised that it would increase the amount of adipose tissue and that the expression of genes associated with thermogenesis, adipogenesis and metabolism would be modified.

Materials and methods - As previously described (2), the offspring of twin-bearing sheep were separated from their mothers at 3 days of age and allocated to one of two dietary groups. The control (C) group was fed milk replacer for 8 weeks and green hay from 14 days of age. The high-carbohydrate high-fat (HCHF) group was fed a 1:1 mixture of milk replacer and cream, plus maize from 14 days of age. At age 6 months, 26 offspring (C: $n=13$; HCHF: $n=13$) were humanely euthanased and

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the sternal and subcutaneous (above the *longissimus dorsi* muscle) adipose tissue depots were excised. The relative expression of genes of interest was assessed by qPCR, using a two-tailed t-test or Mann-Whitney U-test to determine statistical significance.

Results - At 6 months of age, animals on the HCHF diet were heavier (C: 36.1 ± 0.8 kg; HCHF: 42.3 ± 2.6 kg; $p < 0.05$) and possessed more sternal (C: 1.0 ± 0.1 g/kg; HCHF: 4.0 ± 0.2 g/kg; $p < 0.001$) and subcutaneous (C: 1.4 ± 0.1 g/kg; HCHF: 7.2 ± 0.5 g/kg; $p < 0.001$) adipose tissue relative to body weight. There was no detectable expression of genes involved in thermogenesis. A number of genes associated with adipogenesis and metabolism were downregulated in the HCHF group in both tissues (Table 1). Leptin and RIP140 responded differently in the two tissues, being upregulated and downregulated respectively in the HCHF group in subcutaneous (but not sternal) adipose tissue.

Conclusions - Excess energy intake during early postnatal life promotes adiposity and suppresses the expression of genes associated with adipogenesis and metabolism. Conversely, it enhances leptin expression in subcutaneous fat, which may contribute to reduced leptin sensitivity with age.

Gene	Sternal adipose tissue			Subcutaneous adipose tissue		
	C	HCHF	Sig	C	HCHF	Sig
Adipoq	1.00 ± 0.05	0.63 ± 0.10	***	1.00 ± 0.08	0.61 ± 0.08	**
CIDEA	1.00 ± 0.13	0.58 ± 0.16	**	1.00 ± 0.18	0.50 ± 0.06	*
INSR	1.00 ± 0.07	0.57 ± 0.07	***	1.00 ± 0.11	0.54 ± 0.06	**
Leptin	1.00 ± 0.12	0.93 ± 0.07	ns	1.00 ± 0.21	1.95 ± 0.25	**
NR3C1	1.00 ± 0.06	0.60 ± 0.04	***	1.00 ± 0.05	0.69 ± 0.03	***
PPAR γ	1.00 ± 0.03	0.55 ± 0.04	***	1.00 ± 0.08	0.57 ± 0.04	***
RIP140	1.00 ± 0.09	0.86 ± 0.09	ns	1.00 ± 0.07	0.58 ± 0.04	***
SREBF1	1.00 ± 0.11	0.48 ± 0.06	***	1.00 ± 0.07	0.78 ± 0.05	*

Values are mean \pm SEM in arbitrary units. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns not significant at the 5% level.

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PC028

A rat model of snacking and body weight control: Sex differences and limits of compensation after reward consumption

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It is commonly asserted that snacking between meals can lead to weight gain. However, there is little evidence for a positive relationship between snacking and obesity in humans. Our aim was to investigate compensatory behaviour in male and female rats given a palatable, rewarding food snack. We hypothesised that homeostatic systems controlling energy balance would protect from weight gain, initiating compensatory responses such as a reduction in intake of other food sources. Adult male or female Sprague Dawley rats were housed individually in standard conditions with ad lib access to bland food and water. Rats received restricted access to sweetened condensed milk (15 min/day for 10 days). In response to daily reward consumption (~24% of total voluntary daily kcal intake) males compensated accurately by reducing voluntary bland food intake ($19 \pm 4\%$, $n=7$), however, female rats only partially compensated by reducing voluntary food intake ($13 \pm 4\%$, $n=8$). During the reward access period there was no correlation between body weight and total daily kcal intake in males ($R^2=0.0042$, $p=0.64$), but a positive correlation was seen in females ($R^2=0.12$, $p=0.0003$). To test whether there is a limit to compensation in males, rats were presented with three daily food rewards (~56% of voluntary daily kcal intake). Male rats failed to compensate fully, reducing their voluntary food intake by only $35 \pm 7\%$ ($n=7$) leading in turn to an increase in total kcal intake. Despite this increased intake, male rats did not show an increase in body weight and there was no positive correlation between body weight gain and total daily kcal intake ($R^2=0.0018$, $p=0.79$). To test whether learning plays a role in compensation, male rats were presented with irregular food rewards (0-4 rewards/day, presented between 09.00 and 18.00 for 14 days). In this unpredictable access paradigm, rats were still able to compensate fully for reward kcal and maintained a similar body weight to rats fed the same total number of rewards on a regular basis. Our data show that male rats compensate well for small food rewards but females do not and quickly gain weight. The basis for this sex difference is not known. Male rats do not need to learn in order to compensate. Instead, homeostatic mechanisms seem to track total daily kcal consumption despite a reward-driven bias towards consuming high-energy, poor nutrition foods. However, males do not compensate completely for small food rewards. It is possible that homeostatic requirements for other macronutrients drive bland food intake despite the reward-driven overconsumption of kcal.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC029

Feeding patterns regulate metabolic outcome in rodents by modifying ghrelin profiles

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Recent pre-clinical and epidemiological evidence (1,2) suggests that temporal feeding patterns influence metabolic outcome, but the underlying mechanism has been obscured by our inability to control feeding patterns in laboratory animals. To overcome this, we have used a CLAMS-based system to investigate the effect of 3 weeks of grazing (consumption of 0.5g (mice) or 1/24th of the total food intake of ad libitum (AL)-fed controls (rats) every 30 mins during the dark phase (18.00-06.00h)) and meal feeding (MF: three 1hr periods of AL food access at 18.00h, 23.30h and 05.00h). Data cited are mean±SEM, with statistical comparisons performed by 1-way ANOVA and Bonferroni post hoc test.

In 6 week-old male Sprague-Dawley (SD) rats grazing (n=7) and MF (n=6) reduced cumulative food intake (cFI) by 15% (P<0.0001 vs AL-fed controls (n=13)), but only MF reduced body weight gain (Day 18 ΔBW: 153.4±4.5g vs 173.2±3.5g (AL); P<0.05). Grazing elevated proportionate inguinal fat mass by 35% (P<0.01), without influencing epididymal or retroperitoneal fat, with abdominal fat storage efficiency being increased by 26% (P<0.01). MF did not alter adiposity. Tibial length and epiphyseal plate width (EPW) were unaffected by grazing or MF, but grazing shortened the hypertrophic zone by 16% (P<0.01).

In 6 month-old male C57BL6 mice grazing increased cFI by 17% (P<0.01), and elicited a transient increase in ΔBW (0.25±0.62g (n=5) vs -1.06±0.19g (AL; n=6) at day 7; P<0.05). MF reduced cFI by 14% (P<0.05), with a sustained reduction in ΔBW (-2.73±1.12g (n=7) vs -1.01±0.29g at day 14; P<0.05). These effects were abolished in male ghrelin-null littermates (n=5-7).

Given this ghrelin-dependency, we used automated blood sampling to characterise ghrelin profiles in 6 week-old male SD rats catheterised under isoflurane anaesthesia. AL-fed rats (n=9) showed the established (3) mid-light phase peak and late-dark phase nadir in circulating ghrelin (total). Grazing (n=4) and MF (n=4) elicited a

marked anticipatory rise in ghrelin in the late-light phase ($P < 0.01$ vs AL-fed rats), followed by a rapid decline as feeding commenced. However, MF also produced pre-prandial peaks before the mid- and end-dark phase meals ($P < 0.05$), while grazing doubled circulating ghrelin in the last third of the dark phase ($P < 0.05$).

Our data demonstrate that feeding patterns influence fat mass, and that the effects of grazing and MF on weight gain are ghrelin-dependent. Intermittent ghrelin exposure, as produced by MF, is known to promote growth hormone (GH) secretion without elevating fat mass, whereas the temporal alignment of raised ghrelin and feeding activity produced by grazing, suppresses GH secretion and increases fat deposition (4,5). Thus, our data imply that grazing behaviour may contribute to the current obesity crisis.

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PC030

Unacylated ghrelin promotes adipogenesis in rodent bone marrow via the action of ghrelin o-acyl transferase and the growth hormone secretagogue receptor

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Unacylated ghrelin (UAG) accounts for approximately 80% of circulating ghrelin, but does not bind to or activate the receptor for acylated ghrelin (AG), the growth hormone secretagogue receptor (GHS-R) (1). Nevertheless, UAG exerts a distinct spectrum of central and peripheral actions, including the promotion of adipogenesis in bone marrow (2), but not in intra-abdominal white fat (WAT; 3). In the absence of an alternative receptor for this hormone, we investigated the potential interaction of UAG with GHS-R in bone marrow in more detail.

Poster Communications

As previously shown in rats (2), intra-bone marrow (ibm) infusion of AG and UAG (from an osmotic minipump connected to a tibial ibm catheter implanted under isoflurane anaesthesia) induced adipogenesis in wild-type (WT) mice, increasing the number of tibial marrow adipocytes from 22 ± 3 cells/field (vehicle-infused; $n=5$) to 42 ± 1 cells/field (AG; $n=5$; $P<0.001$) and 37 ± 3 cells/field (UAG; $n=4$; $P<0.05$; all data are mean \pm SEM with statistical comparisons performed by 1-way ANOVA and Bonferroni post hoc test). Surprisingly, this effect was abolished in loxTB-GHS-R (GHS-R-null) mice (4), AG- and UAG-treated mice having 100 ($n=5$) and 88% ($n=5$) of the number of marrow adipocytes in vehicle-treated animals ($n=5$). Gas chromatography and mass spectrometry revealed that isolated rat tibial marrow adipocytes contain a range of short-chain fatty acids, including octanoic acid, the substrate used for the activation of UAG. Fluorescence immunocytochemistry revealed that, unlike stomach (which expressed the activating enzyme, ghrelin O-acyl transferase (GOAT), but not the adipocyte-specific marker, PPAR γ) and intra-abdominal WAT (which expressed PPAR γ , but not GOAT), rat tibial marrow adipocytes co-expressed both PPAR γ and GOAT. Immunogold electron microscopy revealed GOAT immunoreactivity in the membrane of the lipid trafficking vesicles and the plasma membrane of rat tibial marrow adipocytes. In addition, the adipogenic effect of a tibial ibm infusion of UAG (using the above method) was completely abolished in GOAT-null mice (5), UAG-treated tibiae ($n=6$) having 117% of the number of marrow adipocytes in vehicle-treated mice ($n=5$), compared to a 120% increase in adipocyte number in WT mice ($n=5,6$; $P<0.05$).

Our data indicate that the adipogenic effect of UAG in bone marrow is dependent upon acylation by GOAT and subsequent activation of GHS-R. This suggests a novel endocrine mechanism, in which the target tissue is able to activate the incoming hormone, by the addition of the activating side-chain. The local expression of GOAT and GHS-R, together with the relative physico-chemical properties of AG and UAG may therefore determine the different activity spectra of these two hormones.

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PC031

Gender differences in weight gain during psychotropic medication treatment and metabolic profile in individuals with serious mental illness

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Aim: The use of psychotropic medications has been correlated with increases in body weight, body fat and waist circumference and related impairments in glucose and lipid metabolism. Hence, individuals with serious mental illness present an increased risk for obesity, metabolic complications and chronic diseases compared to the general population. Significant gender differences have been reported in the pattern and symptoms of mental illnesses, despite no differences in the overall prevalence of mental disorders between genders. However, there is a paucity of research on the effect of gender on the obesity prevalence and metabolic profile of individuals with serious mental illness. The purpose of this study was to examine the effect of gender on weight gain during psychotropic medication treatment and its effects on the metabolic profile of individuals with serious mental illness. **Methods:** 360 individuals with serious mental illness (Males n=89, Females n=271, age: 41.30±12.07 years, BMI: 33.88±6.64 kg/m²) volunteered to participate in the study. Measurements of body height and weight, waist circumference, body composition and basic metabolic rate were performed. Biochemical tests for blood glucose and lipid profile were conducted.

Results: Men exhibited a significantly greater body weight gain during the psychotropic medication treatment (Men:23.60±15.02 vs Women:17.96±12.80 Kgs, P<0.05) despite no significant gender differences in the years of treatment (Men:66.86±64.79, Women:62.37±72.29 months, P>0.05). Men also had a significantly greater body weight, body mass index (BMI), percent body fat and waist circumference compared to females. In terms of metabolic profile, men exhibited greater levels of blood glucose and triglycerides and lower levels of high density lipoprotein cholesterol compared to females (P<0.05). Significant correlations were found between duration of treatment and weight gain, percent body fat and waist circumference (P<0.01).

Conclusions: Men with serious mental illness appear to gain significantly more body weight during treatment with psychotropic medication compared to women, despite the similar duration of treatment. Men also exhibit a more severe metabolic profile, possibly placing them at a greater risk for chronic diseases compared to females. Hence, it is possible that the treatment for serious mental illness needs to be tailored to the gender of the individuals in order to minimize greater risks for obesity and related co-morbidities.

Poster Communications

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PC032

Influence of nutrition on vaginal smear characteristics

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Obesity has important influence of functioning of whole organism. It is believed that both genetic factors and gestational environment contribute to its origin and development (1). There is important influence of maternal nutrition on the physiological and pathological processes in the offspring which is usually explained as posttranslational modification of the nucleic acids of offspring without changing the genetic sequence, which are called epigenetic changes (2). Changes in feeding protocol between generations have influence on metabolic functioning, especially on female reproductive physiology (3). The aim of the study was to determinate impact of mothers' nutrition and nutrition of offspring on characteristics of vaginal smears and lipid levels in offspring.

Nine weeks old female Sprague Dawley rats (n=10) were randomly divided in two groups: high fat diet (HFD, n=5) group which was fed during 6 weeks with high content of saturated fatty acid food and control diet (CD, n=5) group which was given standard laboratory chow during the same period. Offspring from both groups were randomly divided in two subgroups after coupling and lactation period, subsequently there were four groups of offspring with different feeding protocol: CD offspring of CD mothers (n=6), HFD offspring of CD mothers (n=6), CD offspring of HFD mothers (n=6), HFD offspring of HFD mothers (n=6). All rats were weight and blood was collected at the age of 18 weeks, when vaginal smears collection was started during 15 days period. Materials were stained following May-Grünwald-Giemsa staining protocol and observed under light microscope (4). Experiments were approved by the Ethics Committee of the Faculty of Medicine University of Osijek and carried out according to Croatian 'Law for the Care and Use of Animals for Scientific Purposes'.

Groups in which the nutrition protocol between mothers and offspring was changed showed significant changes in characteristics of vaginal smears, whereas the same nutrition protocol between mothers and offspring resulted in normal characteristics of vaginal smears. Significant higher triglyceride level was in HFD-HFD (p=0,001) and cholesterol level in CD-HFD group (p=0,002). All results are

Poster Communications

expressed as mean±standard error (SE). The data was analyzed using Kruskal-Wallis and Mann-Whitney U test. Accepted statistical significance was for $p<0,05$.

Our conclusion is that maternal HFD consumption predisposes offspring to increased risk of estrus disorders which point to the importance of the mother's diet in the regulation of the reproductive cycle of offspring. Given the prevalence on maternal obesity, further studies are needed to evaluate genetic components and to elucidate the complexity of reproductive etiology and pathophysiology.

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PC033

Postprandial effects of cholecystokinin are mediated by brainstem PrRP neurons

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Cholecystokinin (CCK) is a gut-derived hormone that regulates satiety through neural mechanisms. It binds to CCK₁ receptors located on vagal afferents that project to the brainstem and activate circuits in the dorsal-vagal complex. This vagal pathway is critical for CCK-induced satiation, but little is known about the circuitry that is activated downstream. Our lab has shown previously that CCK-induced satiety requires signalling through receptors for prolactin-releasing peptide (PrRP) in the brain⁽¹⁻³⁾, and here we pinpoint the role of a specific population of PrRP-expressing neurons in the nucleus of the solitary tract (NTS) in the brainstem. PrRP neurons are found in three discrete populations in the brain: one in the NTS, another in the ventrolateral medulla of the brainstem and a third in the dorsomedial nucleus of the hypothalamus. To dissect these populations, we first generated a knockout mouse by inserting a lox-STOP-lox (LSL) codon between the PrRP promoter and exon 1 of the gene. Unlike those in the hypothalamus, PrRP neurons in the brainstem are noradrenergic and express the enzyme tyrosine hydroxylase (TH). Thus, expression of PrRP in the brainstem, but not the hypothalamus, was

rescued by crossing LSL-PrRP mice with TH-Cre mice. Subsequent experiments were performed on littermates. All data are presented as mean \pm SEM.

Rescue was confirmed by qPCR and immunohistochemistry. LSL-PrRP mice displayed late-onset obesity and, in contrast to wild-type littermates, did not respond to intraperitoneal CCK with reduced food intake (WT: 0.57 ± 0.05 g vs. 0.24 ± 0.02 g [$P < 0.05$]; LSL-PrRP: 0.44 ± 0.04 g vs. 0.39 ± 0.02 g [$P > 0.05$]; vehicle vs. CCK; $n=6/7$; ANOVA). Restoration of PrRP in the brainstem was sufficient to restore a normal body-weight phenotype in TH-Cre::LSL-PrRP mice. Furthermore, these animals responded to intraperitoneal CCK (food intake: 0.54 ± 0.06 g vs. 0.25 ± 0.02 g; vehicle vs. CCK; $n=6$; $P < 0.05$; ANOVA in same experiment as above). Thus, the brainstem population is sufficient to mediate the satiating actions of CCK.

To show that PrRP neurons in the NTS alone can mediate reductions in food intake, we injected an adeno-associated virus expressing a 'designer receptor exclusively activated by designer drug'⁽⁴⁾ into the NTS of PrRP-Cre mice under 2% isoflurane anaesthesia. This resulted in the transfection of NTS PrRP neurons, permitting us to activate these neurons selectively by intraperitoneal injection of the designer drug, clozapine-N-oxide (CNO)⁽⁴⁾. Activation of PrRP neurons in the NTS resulted in a significant reduction in fast-induced food intake (0.68 ± 0.07 g vs. 0.31 ± 0.06 g; vehicle vs. CNO; $n=10$; $P < 0.05$; student's t-test).

Together our results highlight the importance of central PrRP signalling in regulating body-weight and show, for the first time, that a specific population of PrRP neurons in NTS is necessary for the satiating effects of CCK.

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PC034

Maternal high-fat diet during pregnancy and lactation and effects on male offspring weight and organ development

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Abbreviations: HFD – high fat diet; CD – control diet; IL-6 – interleukin 6; TNF-alpha – tumor necrosis factor alpha

Obesity, as a global epidemic, induces many different disorders like glucose intolerance, type 2 diabetes, dyslipidemia, hypertension or metabolic syndrome. Several studies have tried to explain the correlation between maternal nutrition and consequences in metabolic profile of offspring (1), mainly on an animal model, having

Poster Communications

in mind a hypothesis of a “thrifty phenotype” as an adaptive mechanism preparing the organism to its likely adult environment (2). In this study, we investigated the effects of maternal high-fat diet prior and during pregnancy and lactation on weight, organ development and laboratory findings in male offspring.

Ten female Sprague Dawley rats (*Rattus Norvegicus*), 9 weeks old, were randomly divided in two groups. One group was fed with high-fat diet (HFD group) (3,4), the other with standard laboratory chow (CD group). After pregnancy and lactation male offspring were also randomly divided in two groups each- HFD and CD group. At 12 weeks of age the offspring were anesthetized with pentobarbital (40 mg/kg) and sacrificed by decapitation. Following that blood and tissue samples were collected. From blood samples total cholesterol, triglyceride and glucose level, IL-6 and TNF-alpha were measured. Organs weight: heart, liver, testis, left kidney, spleen, thymus and lungs were measured. Body mass index (BMI) was calculated as body weight (g)/tibia length (cm²). Experiments were approved by the Ethics Committee of the Faculty of Medicine University of Osijek and carried out according to Croatian ‘Law for the Care and Use of Animals for Scientific Purposes’.

We observed an increase in body and organ weight in CD-HFD compared to the CD-CD group, and in all organs except the thymus in HFD-HFD group, but no significant difference in BMI. No significant difference in organ weight between CD-HFD and HFD-HFD group was observed. There was no significant difference in glucose and IL-6 levels between the groups where dams and/or offspring were fed high-fat diet. Total cholesterol level in serum was increased in aforementioned groups. All results are expressed as mean±standard error (SE). The data was analyzed using Kruskal-Wallis and Mann-Whitney U test, a difference was considered significant at $p<0,05$. Our results show that high-fat diet affects organ development and increases cholesterol level.

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PC035

Leptin acts on hypothalamic PrRP neurones to modulate energy expenditure

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The adipokine leptin is essential for maintaining energy homeostasis, and signals via receptors (LepR) in the brain to reduce food intake and increase energy expenditure. While the feeding effects of leptin are well known, the pathways that mediate leptin's effects on energy expenditure are less understood (1,2). Here we show that LepR expression on neurones containing prolactin-releasing peptide (PrRP) is essential for normal energy expenditure in the mouse, and specifically for the effects of leptin on thermogenesis.

Immunohistochemistry revealed that PrRP colocalises strongly with LepR-eGFP in the dorsomedial nucleus of the hypothalamus (DMH) in fluorescent reporter animals. Furthermore, following intraperitoneal (ip) leptin injection, DMH PrRP neurones exhibited a robust increase in STAT3 phosphorylation, indicating a direct neuronal response to leptin. Also, using whole-cell patch clamping of PrRP-eGFP brain slices, DMH PrRP neurones were shown to be inhibited by 100nM leptin (11/18 cells showed a decrease in membrane potential and firing rate).

To study the physiological effects of leptin, a PrRP-cre knock-in mouse was crossed with a floxed LepR mouse (3) (PrRP-cre::LepR^{flox/flox}), to delete LepR solely in PrRP neurones. These crossed animals exhibited obesity, with no difference in baseline feeding, but had decreased oxygen consumption (3069 ± 31 ml/kg/hr vs 2898 ± 56 ml/kg/hr, LepR^{flox/flox} vs PrRP-cre::LepR^{flox/flox}, $n=5/4$, $p<0.05$; values are means \pm SEM, compared by ANOVA). The mice also had lower core-body temperature as measured by a temperature transmitter implanted under 2% isoflurane anaesthesia ($36.83 \pm 0.08^\circ\text{C}$ vs $36.57 \pm 0.10^\circ\text{C}$, LepR^{flox/flox} vs PrRP-cre::LepR^{flox/flox}, $n=6$, $p<0.05$). Furthermore, ip injection of leptin caused a sustained increase in body temperature of $\sim 0.5^\circ\text{C}$ for the 3 hours post-injection in LepR^{flox/flox} mice ($n=6$, $p<0.001$), but had no effect in PrRP-cre::LepR^{flox/flox} animals.

Finally, under 2% isoflurane, an adeno-associated virus (AAV) containing a cre-dependent anterograde tracer was injected into the DMH of PrRP-cre mice. Infected PrRP neurones showed local projections within the DMH and substantial projections to the paraventricular nucleus of the hypothalamus (PVH). Additionally, a retrograde tracer injected into the PVH colocalised with PrRP-eGFP neurones in the DMH.

In summary, leptin acts directly through LepR on DMH PrRP neurones to control energy expenditure and thermogenesis. Furthermore, DMH PrRP neurones project predominantly to the PVH, a major site of integration and processing of autonomic functions.

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PC036

Renal denervation improves blood pressure, renal function and renin angiotensin system in neonatal hyperleptinaemic rats

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Over-activation of the renin-angiotensin-aldosterone system (RAAS) plays an important role in the pathophysiology of hypertension. In rodent models, juvenile offspring of obese dams exhibit sympathetic mediated hypertension and hyperphagia, increased adiposity and insulin resistance in adulthood which is associated with an exaggerated leptin surge in early postnatal life(1). We aimed to elucidate the mechanisms underlying RAAS and oxidative stress and the beneficial effects of renal denervation (RD) in a neonatal hyperleptinaemic rat model.

Male and female Wistar rats were given intraperitoneal injections of leptin twice daily (L-Tx, 3mg/kg) from postnatal day 9 to 14, to mimic the exaggerated leptin surge in neonatal offspring of obese dams, versus saline treated (S-Tx). At 22 days of age, rats were subject to telemetry surgery performed under 3% isoflurane (inhaled) and probes were inserted into the arch of the aorta via the left carotid artery. After 7 days of baseline recordings each group was further divided into a RD group and a sham operated (SH) group to examine the effects of RD on blood pressure. RD was performed under isoflurane through an incision in the dorsal midline and all visible nerves were cut and painted with 10% phenol. RD was confirmed if noradrenaline content of renal tissue was <10% of the mean value in SH. The right kidneys were excised from sacrificed rats and stored at -80°C for gene expression analysis with all qPCR results normalised against the geometric mean of the housekeeper genes HPRT and TATABOX according to the geNorm method. Statistical analysis was performed using two-way ANOVA tests to compare neonatal treatment with surgery; significant results were those with a P value of <0.05. At 30 days of age, neonatal leptin rats demonstrated increased blood pressure, enhanced RAAS and decreased creatinine clearance [GFR, ml/min/kg, L-Tx, 2.8±0.1 vs. S-Tx, 3.5±0.2, n=6, p<0.05]. RD reduced 24-h mean arterial pressure (MAP) in male (L-Tx-SH, 114±2 mmHg vs. L-Tx-RD, 99±1 mmHg, P<0.0001, n=4-5) and female (L-Tx-SH, 115±3 mmHg vs. L-Tx-RD, 96±3 mmHg, P<0.0001, n=4-5) rats and improved renal function. Furthermore, RD normalised cortex expression of type-1a angiotensin II receptor (AT1a) in L-Tx rats which was increased 100-fold in females and 4-fold in males compared to S-Tx. Aquaporin-2 (AQP2) has also been

associated with angiotensin II induced hypertension and reactive oxygen species(2) and we observed reduced AQP2 expression concomitant with increased NADPH oxidase 4 (NOX4) expression.

Thus RD significantly reduces angiotensin II induced hypertension, improves renal function and normalises the RAAS. These findings lend evidence to support the exciting prospect of RD as a future target for the treatment of hypertension in select population groups.

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British Heart Foundation

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PC037

Loss of hepatic dimethylarginine dimethylaminohydrolase-1 causes increased fat deposition

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Obesity is a major health and economic burden. It is becoming increasingly common and is associated with serious and life threatening secondary complications, predominantly involving the cardiovascular system.

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthases. Increased levels of plasma ADMA are associated with multiple pathologies; it is an established biomarker of cardiovascular disease and has been associated with obesity, type 2 diabetes, and insulin resistance. However at present little is understood about the direct effects of ADMA on lipid accumulation.

Dimethylarginine dimethylaminohydrolase (DDAH) catalyses the metabolism of ADMA, there are two isoforms of the enzyme which are both involved in the control of ADMA and NO. In this study we show that hepatocyte specific knockout of *ddah1* causes increased fat deposition.

Adult hepatocyte specific *ddah1* knockout mice and their wildtype counterparts were put on a 60% high fat diet for 12 weeks. On normal diet adult mice showed no difference in weight, fat mass or glucose handling and had no obvious liver dysfunction. Following high fat diet, hepatocyte *ddah1* knockout mice have a significantly higher fat mass (wildtypes; $8.31 \text{ g} \pm 1.089$, hepatocyte *ddah1* knockout; $17.66 \text{ g} \pm 1.299$) and overall weight (wildtypes; $41.47 \text{ g} \pm 1.788$ hepatocyte *ddah1* knockouts; $51.9 \text{ g} \pm 1.149$). Glucose tolerance tests showed gross impairment in

response to a bolus of glucose and fasting glucose levels were significantly different between wildtypes and knockouts (wildtypes; $7.133 \text{ mmol/l} \pm 0.333$, hepatocyte ddah1 knockouts $9.20 \text{ mmol/l} \pm 0.5797$). Indirect calorimetry was used to assess the metabolic rate of animals post high fat diet and showed hepatocyte specific ddah1 knockout animals to have a lower metabolic rate but no significant difference in activity or food intake.

ADMA has been linked to increased mTOR gene expression and lipid biosynthesis in adipocytes. As a result we investigated gene expression in the liver of animals following high fat diet. Hepatocyte specific ddah1 knockouts showed significantly increased gene expression of both mTOR and SREBP1c.

These data suggest that knockout of hepatic ddah1 and consequent increases in ADMA are novel regulators of lipid synthesis and weight gain on a high fat diet.

MRC

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PC038

The role of microalbuminuria as a predictor of gestational diabetes in obese pregnant women from the UK Pregnancies Better Eating and Activity (UPBEAT) pilot trial

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Introduction - Albumin:creatinine ratio (ACR), a marker for endothelial dysfunction, is an established independent predictor for cardiovascular and renal disease. Recent evidence suggests that a relationship also exists between obesity, early insulin resistance and microalbuminuria. However, the role of ACR as a predictor of gestational diabetes (GDM) in obese pregnant women is unknown.

Aim - To explore the role of ACR in the prediction of GDM in obese pregnant women participating in the UPBEAT pilot study.

Methods - Fasting mid-stream urine samples were obtained from 63 obese pregnant women participating in a pilot study of a complex intervention of dietary advice and physical activity recruited at 15+0 to 17+6 weeks. Demographic characteristics were recorded at recruitment. Albumin (mg/L) was analysed by laser immunonephelometry on a Siemens BN Prospec and creatinine (mmol/L) by stable isotope dilution tandem mass spectrometry in spot urine samples. Univariate analyses were performed to determine independent predictors for GDM and an area under the receiver-operating curve (ROC) was calculated for the model.

Results - Of the 63 participants, 39.7% (n=25) developed GDM. Participants with GDM were significantly older (34.2 vs 30.6 years; $p = 0.01$), with lower parity ≤ 1 (68% vs. 32%; $p = 0.004$) and tended to be black in comparison to those without GDM. ACR at 15+0 to 17+6 was independently associated with GDM in univariate analyses (0.92 g/mol vs 0.73 g/mol; $p = 0.012$). The area under the receiver-operating curve for the prediction of GDM for clinical factors, significant within the univariate analyses alone was 0.70 (CI 0.56 to 0.85) and increased further with the addition of ACR at 15+0 to 17+6 (ROC AUC 0.77; CI 0.65 to 0.89; $\chi^2 = 0.16$). **Conclusions** - These findings suggest there is an association between early second trimester urine ACR and subsequent development of GDM in obese pregnant women. Routinely recorded clinical data with ACR measured in early pregnancy may add to the known biochemical predictors of GDM. Further study in a large prospective cohort is now required, in order to develop a panel of predictive markers for GDM with high predictive performance suitable for clinical practice.

Pilz S et al. (2014), *Diabetes Care* 37,1597-603

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PC040

Incidence of obesity, maternal respiratory, metabolic and obstetric outcomes evaluated between 2000-2014

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Increasing UK obesity includes expectant women¹; the Confidential Enquiry into Maternal and Child health (2000-2) showed that 35% of all women who died were obese, compared with 23% of the general population. Risks for babies of obese women include stillbirth, macrosomia, prematurity and neonatal death². We wished to determine the change in incidence of obesity in pregnant women, associated maternal disorders and the obstetric sequelae for women in our clinic. The electronic database of York Teaching Hospital antenatal department was interrogated to establish the number of obese women (**O**, body mass index (BMI) >30 Kg/m²) and super obese (**SO**, BMI >50 Kg/m²) women who booked in to the clinic and who delivered. The incidence of **O** and **SO** rose between 2001 and 2011 (**Fig.1**). Using the database, we identified all 6 **SO** who presented in 2011 and matched them against 6 non obese **N** (BMI <30) women who delivered the same day (Total annual deliveries 3391, 3826 women booked in 2011). The proportion of asthma (67% vs 19%), diabetes (50% vs 0%), and depression (50% vs 17%) was higher in **SO** than **N** (BMI <30). A further 30 **O** and 20 **N** women were identified and evaluated; 16 **O** and 0 **N** had impaired glucose tolerance. Mean(range) resting oxygen saturation supine awake at rest was 99(98-100)% in **N** and 98(97-100)% in **SO** & **O** ($p = 0.016$, unpaired t-test). Snoring was seen in 67% **SO** & **O** and 40% **N** ($p = 0.07$, χ^2). No dif-

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ference was seen in the mean Epworth sleepiness scores in these patients (6.2 in **O**, 6.6 in **N**) or mean(range) heart rate (84.7(72-106) bpm in **O** and **SO**, 81.5(70-99) bpm in **N**), indicating that these measures are insensitive in distinguishing these patients. Both maternal systolic and diastolic blood pressure increased with BMI at booking and at 36 week gestation. Mean(range) weight gain during pregnancy was 6.3 (-5 - 20)kg in **O** and **SO** and 10(1-16)kg in **N**, indicating some effectiveness of antenatal care. Only 67 % **SO** delivered babies in an acceptable weight range (birth weight 2.7-4Kg) vs. all **N**. 37.5% **O** and **SO** had miscarriages vs 8.3% **N** ($p=0.066$, χ^2). In 2014, a further 7 **O** (4 caesarean births) and 7 matched **N** (2 caesarean) were evaluated. Obesity has a significant and increasing burden on the respiratory and metabolic health of mothers and adversely affected obstetric outcomes.

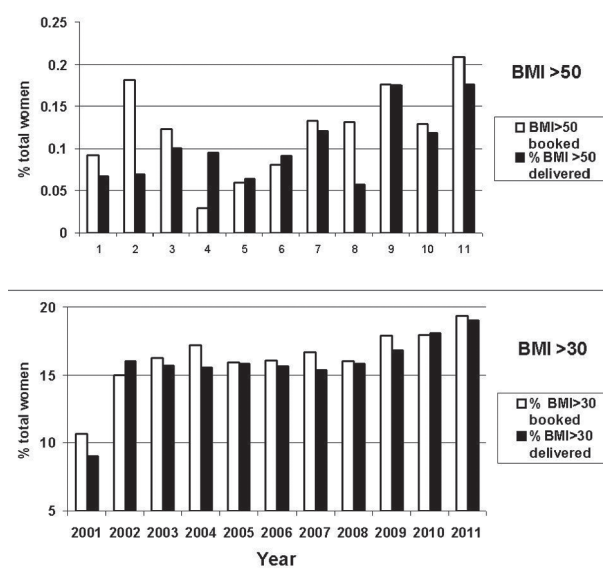


Fig.1 Percentage of SO (upper panel) and O (lower panel) as a proportion of total women at booking (open bars) and delivered (filled bars) at York Teaching Hospital between 2001 and 2011.

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PC041

Investigation on the effect of alcohol and cannabinal on hypothalamic pituitary gonadal system

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This study investigated the effects of oral administration of alcohol and cannabinal on the hypothalamic pituitary gonadal system in male adult rats. Twenty five male rats were divided into five groups containing five rats each and were treated for a period of 48 days via oral administration. Group one serves as the control, group two was administered 5mg/kg body weight methanol, group three was gavaged with 3g/kg body weight as 25%v/v alcohol, group four was gavaged with 10mg/kg body weight cannabinal and group five was treated with alcohol (3g/kg body weight as 20%v/v) and cannabinal (10mg/kg body weight). At the end of the experimental period, blood was collected via the retro-orbital sinus under ether anaesthesia and was allowed to clot for hormonal assay and the brain was dissected and immediately fixed. Semen analysis was carried out by exposing the testis together with the epididymis and the epididymis was carefully separated and caput was removed. The caput was then transferred unto a pre-warmed slide and lacerated to release some semen unto the slide surface. The animals were sacrificed and their reproductive organs were removed and weighed immediately. There was no significant change in the body weight but there was a significant change in the percentage weight difference in the experimental groups when compared with the control group. Serum level of testosterone of the groups treated with alcohol, cannabinal, alcohol plus cannabinal were significantly decreased (p0.05) when compared with the control rats. However, there were reduction in sperm motility and sperm count of rats exposed to alcohol, cannabinal, alcohol plus cannabinal treated rats in comparison to the control rats. The histological section showed alteration in the hypothalamic and testicular cytoarchitecture in groups treated with alcohol or/and cannabinal treated rats, while there was reversal of this in the groups co-treated with quercetin. The results suggest that alcohol and cannabinal administration have deleterious effect on male reproductive activities (system) in rats.

Keywords: alcohol, cannabinal, HPG-axis, histomorphology, sperm content, hormone profile

Poster Communications

Effects of alcohol and cannabinal on sperm profile of experimental rats treated for 48 days

Treatment	Sperm motility(%)	Sperm count (x106/ml)	Abnormal sperm morphology (%)
Control	93.60±1.56ab	17.00±1.16a	6.29a
Methanol	85.00±2.23ab	20.00±0.97a	7.43a
Alcohol	40.00±1.58a	4.40±0.51b	23.10ab
Cannabinal	35.00±1.87a	4.80±0.37b	26.95ab
Alcohol+cannabinal	37.00±1.12a	4.20±0.85b	26.17ab

Data are represented as mean±S.E.M. Values with different superscript in the same column are significantly different.

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PC042

Human placental taurine transporter activity is reduced in pregnancies complicated by gestational diabetes mellitus

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INTRODUCTION: In the UK it is estimated that 20% of pregnant women are obese (body mass index BMI≥30kg/m²) at their first antenatal appointment. Maternal obesity increases the risk of gestational diabetes mellitus (GDM), a condition of severe insulin resistance and hyperglycemia. Both GDM and maternal obesity increase the risk of fetal mortality and morbidity and are associated with elevated placental oxidative stress. Animal studies have shown that taurine supplementation in pregnancy protects against diabetes-induced oxidative stress in mother and embryo¹. We have shown that the activity of TauT, the protein that transports taurine into the placenta, is significantly lower in obesity than in normal pregnancy². Taurine is an antioxidant amino acid and a reduction in placental

taurine uptake could contribute to elevated oxidative stress in obesity and GDM. Here we test the hypothesis that placental TauT activity is lower in women with GDM as compared to normal pregnancy. METHODS: Placentas were collected at term from women with GDM and women experiencing normal pregnancy (NP). BMI was recorded at antenatal booking (12 weeks gestation) to allow separation of women into obese (BMI \geq 30) and non-obese (BMI 18.5-29) groups. TauT activity was determined by measuring Na⁺-dependent uptake of ³H-aurine into freshly isolated placental villous tissue over 120min (fmol/mg protein). TauT protein expression in membrane-enriched placental homogenates was assessed by Western blotting. Nitrocellulose membranes were re-probed for β -actin to normalise TauT expression. Data are presented as medians and analysed by Mann Whitney. RESULTS: In non-obese women, placental TauT activity was significantly lower in GDM compared to NP (2335 and 4137 respectively, $p<0.03$, $n=7$ in each group). In common with our earlier observations², in NP TauT activity was lower in placentas of obese (2590, $n=3$) compared to non-obese women (4137, $n=7$). However, TauT activity did not differ in obese ($n=6$) and non-obese ($n=7$) women experiencing GDM ($p>0.05$). Following Western blot analysis, TauT protein was observed at 70kDa consistent with previous reports in human placenta². In non-obese women, there was no difference in the normalised density of immunoreactive signals between NP and GDM ($n=7$ in each group). CONCLUSIONS: Placental TauT activity, but not expression, is lower in non-obese women with GDM compared to NP. We have previously shown that the reduction in TauT activity in obesity is not associated with a change in protein expression², suggesting that post-translation regulation of transporter activity is common to both conditions. Experiments are ongoing to determine the mechanism of TauT down-regulation and to explore a link between reduced TauT activity and elevated placental oxidative stress in obesity and GDM. Shivananjappa MM & Muralidhara M (2012) *Reprod Biomed Online* 5,558-66.

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PC043

Methodological improvements in infrared thermography for the *in vivo* analysis of brown adipose tissue

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Background - The emergence of brown adipose tissue (BAT) as a possible novel obesity prevention and treatment target has led to renewed interest in its physiology. The lack of an acceptable, safe, non-invasive method for repeated analysis is a significant barrier to *in vivo* human studies as the use of PET-CT in large prospective studies is limited by expense and ionising radiation exposure.

We have developed a novel technique for measuring BAT activity using thermal imaging (TI)[1]. TI is a cheap, quick, non-invasive, valid and reproducible procedure acceptable to children as young as five years old[2]. We present here novel modifications to our original method.

Method - As previously described[1,2] images centred on the anterior aspect of the neck were obtained using a thermal imaging camera (FLIR B425; FLIR Systems, Sweden) over a 25 minute period, at a rate of 6/minute. Following a period of acclimatisation, the participant's right hand was submerged in cold water ($18^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$), an effective stimulator of BAT activity[1]. After 10 minutes of stimulation with cold water, each participant removed their hand and imaging was continued for a further 8 minutes.

Images were exported using ThermoCAM Researcher Pro 2.10 (FLIR systems) and analysed using a custom-designed code in MATLAB (Mathworks, USA). Key points on each image were identified to define the region of interest (ROI) within which upper temperature percentiles were calculated. Data were exported from MATLAB to Excel 2010 (Microsoft Corporation, USA). Moving averages of order 7 were calculated. A moving average replaces each point with an average of the point and the points on either side, thereby reducing the effect of random fluctuations. **Results** - Approximately 150 images per participant were captured. Time series were plotted using the original method (Fig 1) compared with the increased image frequency and moving averages (Fig 2).

Conclusions - Previous data have shown an increase in the average temperature of the upper percentile of temperature in the region of interest (ROI) over the anterior aspect of the neck with cold water stimulation. Within this, there is a degree of natural variation in measurements. We show here that utilisation of a simple moving average substantially reduces point-to-point fluctuation revealing a clear trend line.

By increasing the number of images taken per minute from 1-6, we are able to use higher order moving averages. Previously, the ROI had to be defined for each image using the irregular polygon tool within the ThermoCAM software, which is a time-intensive process. Our new semi-automated process allows larger numbers of images to be processed consistently and quickly.

In summary, thermal imaging is an essential tool in the in vivo analysis of BAT, particularly in human participants and the methodological improvements outlined above further improve the method.

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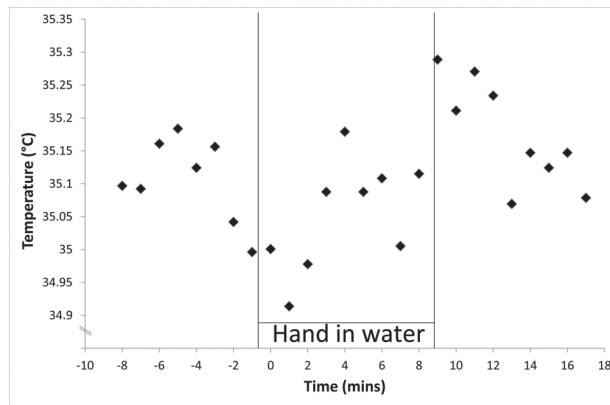


Figure 1. Times series of BAT temperatures using an image capture rate of 1 per minute.

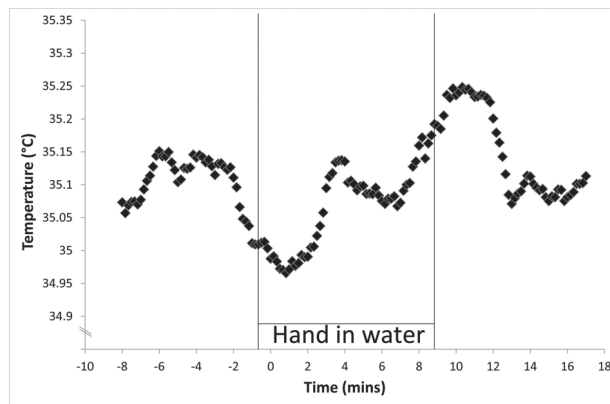


Figure 2. Times series of BAT temperatures using an image capture rate of 6 per minute and applying a moving average of order 7.

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PC044

Fat but fit: Regulation of adipokine production and adipose tissue function in an animal model of obesity and extreme weight change

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Obesity is characterised by large fat depots, dysregulation of adipose tissue and increased visceral fat. Seals are informative models for the study of obesity because they a. are up to 45% body fat (1) with 'diabetic' metabolic profiles (2); b. undergo substantial mass changes throughout their lifecycle - pups triple in mass as they deposit fat while suckling (3) - and c. store fat almost exclusively as subcutaneous blubber. However, regulation of their fat storage and utilisation is poorly understood. Here we tested the hypothesis that changes in gene expression of adipokines, which contribute to fat balance in humans, are regulated by physiological state or body mass in naturally feeding and fasting grey seals. Using ex vivo blubber explants, we also tested the hypothesis that adipokine and lipase gene expression are altered by high glucose, free fatty acids and glucocorticoids (GCs) in seals, as in humans and rodents. Handling procedures were performed under Home Office project licence 60/4009 and conformed to the UK Animals (Scientific Procedures) Act. Grey seal pups (n = 10) were captured early (day 5) and late (day 15) during suckling, and the same pups were captured again early (day 5) and late (day 15) during their postweaning fast. Blubber samples were taken using standard techniques and appropriate general (0.01ml/ 10kg zoletil100 TM i.v.) and local (2ml 2% w/v lignocaine (Lignol) s.c.) anaesthesia (4). Blubber leptin and adiponectin gene expression were measured in triplicate using qPCR. Mean CT value for each gene was normalized to S9, expressed as fold difference relative to the early suckling sample for each animal using the Pfaffl method (5) and analysed in R using linear mixed effects models (LME). Blubber leptin and adiponectin mRNA abundance did not change during feeding or fasting, and was not related to body mass (LME: $p > 0.05$). Blubber samples from adult grey seals (n=10), captured using tangle nets at Abertay Sands and anaesthetised with 0.05ml/ 10kg zoletil100 TM i.v. and Lignol, were washed in Krebs Ringer solution, minced and incubated overnight in media supplemented with high glucose (25mM); palmitate (100mM) or hydrocortisone (500nM). Exposure to palmitate or hydrocortisone had no effect on abundance of leptin, adiponectin, adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL) mRNA expressed relative to CycA (REST 2009; $p > 0.05$). ATGL mRNA abundance tended to increase in high glucose media (REST2009; $p = 0.076$). Our data suggest key functional genes are not regulated in seal blubber as they are in human fat. Our findings and approach may be informative in elucidating mechanisms that control subcutaneous fat deposition in mammals. We hope to promote discussion of the utility of this animal model of extreme fat accumulation in the study of human obesity.

Poster Communications

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Royal Society; SNH

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PC045

Active videogames can represent the actual intensity avoiding sedentary lifestyle and obesity? A reliability study

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Sedentary lifestyle remains a serious clinical problem and lower activity level may be a start to developed or aggravate no chronic degenerative diseases such as obesity. The American College of Sports and Medicine recommends daily cardiovascular activity 30-60 minutes with moderate intensity (50-69% heart rate) (Donnelly et al., 2009; Garber et al., 2011). Several interventions have been proposed to change sedentary habits, one of them are the active videogames (Brito-Gomes et al., 2014). They can be unstructured, and used to recreation or rehabilitation. And can be structured to raise heart rate, oxygen consumption and levels of physical activity. The main of this study was to compare the intensity level of the physical exertion between the active videogame (AVG) structured and unstructured. The present study has a positive appreciation of the University of Pernambuco (UPE) Research Ethics Committee. 8 young male adults (19±0.9 years, 23.1±2.4 kg /m.m) without gaming experience and no physically active performed on the Moment 1 measurements: anthropometric (weight and height), hemodynamic (resting heart rate) and oxygen consumption with maximal test on a cycle ergometer (Cateye, EC-1600, Ergociser, Japan) following the ACSM with the protocol of Astrand-Ryhming (1954) and metabolic gas analyzer (CPX/D, Cortex, Germany). After 48 hours, at the Moment 2, it was randomized for the groups the 2 types of AVG sessions: 1) Kinect Sports modality: Boxing (unstructured) and 2) Nike Kinect Training (structured). Each session lasted 30 minutes and the heart rate was recorded by a heart rate monitor (Polar, FT1, Finland). To analyze the heart rate of the test and the oxygen consumption at each AVG it was made a Spearman's Correlation. Wilcoxon-paired test was performed to analyze the difference between the percentage of heart rate reached for each AVG and percentage of heart rate

tested. It was used a significance level of 5% ($p \leq 0.05$). The heart rate and oxygen consumption on Boxing and Nike Kinect shows a strong correlation with values of 0.98 and 0.99, respectively. For the Boxing group was verified a percentage of the heart rate reached between 58 to 79 and a percentage of heart rate tested between 59 to 100 ($p=0,068$). In the Nike Kinect group, it was verified a percentage of the heart rate reached between 92 to 99 and a percentage of heart rate tested between 98 to 112 ($p=0,068$). Thus, the structured AVG shows greater enhance of the percentage's heart rate. Knowing that the heart rate have a strong correlation between the oxygen consumption, this data suggest that structured AVG may be a good tool to exercise and probably, reduce sedentary lifestyle. Also, if used in obese, perhaps it should avoiding the obesity status.

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We thank all members of the Evaluation in Human Performance lab - UPE / esef - Brazil

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PC047

Visceral adiposity and its differential impact on systemic and cerebrovascular function

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Introduction: Traditionally, much interest has focused on the cardiovascular complications of obesity while its impact on the cerebrovasculature is comparatively less well established. This is surprising given its association with stroke, cognitive decline, dementia and depression which ultimately decrease an individual's functional quality of life (1). To explore this in more detail, the present study was designed to examine to what extent abdominal obesity and the excessive accumulation of visceral adipose tissue (VAT) is associated with impaired cerebrovascular function in the form of cerebral hypoperfusion and decreased vasoreactivity.

Method: We recruited 10 non-medicated obese aged 28 (mean) \pm 8 (SD) years and 13 non-obese participants (22 \pm 5 years). Total body mass (TBM), body fat % (BF) and body mass index (BMI) were collected via bioelectrical impedance analysis (BC-

418, Tanita) and VAT assessed using computer tomography scans (GE Hawkeye Scanner). Aortic (carotid to femoral) pulse wave velocity (PWV) was performed via applanation tonometry (SphygomoCor BPAS-1). Middle cerebral artery velocity (MCAv, transcranial Doppler Ultrasound), mean arterial pressure (MAP, finger photoplethysmography) and end-tidal carbon dioxide (PETCO₂, capnography) were measured during steady-state exposure to hypercapnea (5% CO₂ in balanced air) and hypocapnea (hyperventilation) as previously outlined (2). Cerebrovascular resistance (CVR) and conductance (CVC) were calculated as MAP/MCAv, and MCAv/MAP respectively. Cerebrovascular reactivity (CVRCO₂) was calculated as the percent change in MCAv per mmHg change in PETCO₂. Data were analysed using a 2-way repeated measures ANOVA and independent sample t-tests to establish between group differences. Correlations between VAT and haemodynamic variables were performed using Pearson Moment Correlations, with significance established at $P < 0.05$.

Results: By design, obese had a higher BM (119.3 ± 34.9 vs. 69.7 ± 2.3 kg, respectively), BF (29 ± 6 vs. $22 \pm 8\%$), BMI (36.9 ± 9.0 vs. 24.2 ± 2.9 kg.m²) and VAT (81 ± 27 vs. 45 ± 23) compared to the non-obese controls ($P < 0.05$). Aortic PWV was shown to be elevated in the obese (6.8 ± 1.0 vs. 5.8 ± 0.9 m/s, $P < 0.05$) and associated with VAT ($r = 0.41$, $P < 0.05$). In contrast, we failed to observe any between group differences in cerebral haemodynamic function.

Conclusions: Collectively, the present findings demonstrate that while excess visceral adiposity is associated with impaired systemic vascular function, such adverse effects fail to “translate” to the cerebrovasculature which appears comparatively more “preserved”. The mechanisms and indeed long-term clinical consequences associated with the differential impact of VAT on the human circulation warrant further investigation.

Table 1: Cerebral haemodynamic function

		MCAv (cm/s)	MAP (mmHg)	CVR (mmHg/cm/s)	CVC (cm/s/mmHg)	PETCO ₂ (mmHg)	CVRCO ₂ (%/mmHg)
Obese (n=10)	Baseline	62±15	91±9	1.54±0.40	0.69±0.21	38±4	
	Hypercapnea	82±19	94±9	1.22±0.30*	0.87±0.25	49±4	
	Δ(%)	32±11	4±6	-20±4	26±7	28±8	
	Baseline	63±17*	93±10	1.57±0.44	0.68±0.19	37±3	
	Hypocapnea	47±17*	86±11	1.97±0.62	0.55±0.18	24±5	
	Δ(%)	-26±8	-8±5	25±10	-20±7	-34±16	
	Range	58±14	14±7	46±13	46±12	62±18	5.73±1.77
Non-obese (n=13)	Baseline	64±11	77±22	1.23±0.37	0.99±0.69	38±3	
	Hypercapnea	88±13	86±18	0.98±0.20	1.07±0.24	48±3	
	Δ(%)	38±11	5±4	-23±7	25±28	29±8	
	Baseline	65±9	85±14	1.32±0.21	0.78±0.12	36±3	
	Hypocapnea	44±7	76±17	1.76±0.46	0.64±0.33	25±4	
	Δ(%)	-32±6	-11±15	32±27	-19±27	-31±7	
	Range	70±11	16±14	60±18	63±23	60±11	6.53±1.23

Notes: Values are mean±SD; * different ($P < 0.05$) vs. non-obese; Δ(%), delta change.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC048

Body adiposity: Changes during the menstrual cycle

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The menstrual cycle (MC) is a phenomenon that affects women between 13 and 50 years old, approximately. Which has a infradian pace with fluctuations in blood concentrations of ovarian hormones, which induce morphological, metabolic, behavioral and emotional changes. The objective of this study was to analyze the behavior of body fat (%G) during the menstrual cycle. Collecting four sessions were conducted at four different times during a menstrual cycle, according to the division found in Gonçalves et al. (2011). The first in the menstrual phase (between the 1st and the 5th day of MC), the second in the follicular phase (between the 6th and the 11th day of the MC), the third in the periovulatory phase (between the 12th and the 16th day of the MC) and the last in the luteal phase (between the 17th and the last day of the MC). In each collection sessions took place the measurement of skinfolds (Lange, USA) in the following regions: biceps (BP), mid-axillary (AX), supra-iliac (SI), abdominal (Ab), subscapularis (SE), triceps (TP), thigh (CX) and leg (PE). Measures of all points were performed in triplicate and on the right hemisphere, according to the standardization of ISAK (2011). By performing measurements of the skinfolds of 15 university eutrophic and eumenorrheic, we carried out a Spearman correlation between measurements of skinfolds and the production of ovarian hormones in the literature (Halbe, 1993) and a high correlation of midaxillary skinfold was observed ($\rho = -0.949$, $p = 0.05$) with the production of progesterone, which is a measure widely used in equations to estimate body density and subsequently the percentage of fat. After, the body density was estimated by equations that uses the midaxillary skinfold, and subsequently the %G, by the Siri (1961) equation. To classify them as a health risk, some had three different classifications during the cycle. Given the findings, it is concluded that equations using the midaxillary skinfold should be avoided, as well as body composition assessments should always be conducted in the same phase of the menstrual cycle. In addition to state the menstrual phase, to carry out the measures, by presenting the least rate of production of ovarian hormones.

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PC049

Lipid profile changes in adipose tissue as a predictor of puberty and inflammation in Large White pigs

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Darwin in his book "The Descent of Man and Selection in Relation to Sex" (1871) observed that there are some characteristics that do not appear to help an organism adapt to its environment and are thus not explained by natural selection [1]. He suggested that they feature in the process of sexual selection. The induction of important traits through sexual selection and domestication such as variations in lean mass and muscular growth [2-4] are often accompanied by undesirable metabolic alterations, such as neonatal mortality and reduction of reproductive capacity [2,3]. The intensive selection pressure applied to commercial porcine breeds to increase protein accretion is accompanied by early decline in fertility and adipose tissue dysfunction [3,4]. However, the metabolic mechanisms underlying these processes in pigs are still not completely characterised. Utilizing a comprehensive lipidomics platform, we compared metabolic changes in adipose tissue of Large White pigs.

Visceral (VAT) and subcutaneous adipose tissue (SCAT) were collected after humane euthanasia from 7 day (n=7, (SCAT only)) and 6 month (n=8 (both depots)) old pigs. Fatty acid (FA) profiles and gene expression of SCD-1, FASN (lipogenic genes), IL-6 and MCP-1 (pro-inflammatory genes) were assessed by gas-liquid chromatography and real-time PCR, respectively. Lipidomics data were analysed using Bayes moderated t-statistics, unsupervised hierarchical clustering and principal component analysis (PCA) using ArrayMining version 1.0 [5]. Gene expression analysis was evaluated by applying General Linear Models using SPSS 16.0.

The SAT FA profile was acutely affected by the transition to puberty, resulting in twenty-two significant changes in FA content ($p < 0.05$), most of those increasing with age were saturated FAs including C14:0, C18:0 and C22:0. Comparative analyses of VAT and SCAT in six month old pigs identified significant changes in 13

fatty acids, with saturated fatty acids increasing only in VAT ($p < 0.05$). Increasing age activated the transcription of pro-inflammatory genes (IL-6 and MCP-1) but decreased the expression of lipogenic genes (FASN and SCD-1) ($p < 0.05$) in both depots, with more pronounced effects in VAT. Both clustering analyses identified the visceral depot as an independent cluster at 6 months, in relation to the other two depots.

In this breed, increasing age leads to a shift in lipid profile, suggesting that an enhanced presence of saturated FAs induces expression of pro-inflammatory cytokines, particularly in the visceral depot.

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PC050

Melanocortin 2 receptor accessory protein 2 (Mrap2) is associated with obesity and mediates hypothalamic melanocortin-4-receptor trafficking in vivo

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Rare loss-of-function mutations of melanocortin-2-receptor accessory protein 2 (MRAP2) have been associated with severe, early-onset obesity in humans. Whole body deletion and targeted brain specific deletion of the Mrap2 gene resulted in severe obesity in mice [1]. In vitro data have shown Mrap2 interaction with the melanocortin-4-receptor (MC4R) affecting receptor signalling as a consequence. However, the mechanism by which Mrap2 regulates body weight in vivo is less well understood with differences between Mrap2 and Mc4r knockout (KO) mice

phenotypes. In this study we show that, consistently with the previous work, Mrap2 KO mice have severe early obesity without detectable changes to food intake or energy expenditure. To further investigate the *in vivo* role of Mrap2 we used a plasma membrane enrichment technique to look at the hypothalamic Mc4r protein surface expression in the mutant mice compared with the wild-type. It was found that Mrap2 KO caused a significant decrease of the levels of Mc4r protein in the plasma membrane fraction suggesting reduced trafficking of the receptor to the cell surface. Thus, our data suggest that *in vivo* Mrap2 mediates hypothalamic Mc4r surface expression and taken together, this work corroborates the role of Mrap2 in obesity as a regulator of melanocortin pathway.

Asai et al, Science 2013

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PC051

Arterial hypoxaemia and appetite control in humans; focus on free radical formation and its impact on peptide YY and pancreatic polypeptide metabolism

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Background and Aims: To what extent arterial hypoxaemia (AH) influences the neuroendocrine regulation of energy homeostasis remains unclear. We examined if AH causes anorexia subsequent to a systemic elevation in the circulating plasma concentration of peptide YY (PYY) and pancreatic polypeptide (PP). We further reasoned that these increases would be associated with an elevated regional (CSF) formation of free radicals given that hypothalamic reactive oxygen species suppress appetite through activation of satiety-promoting melanocortin neurons (Diano et al., 2011).

Methods: Twenty-two subjects were randomly exposed to both 12% (normobaric hypoxia) and 21% oxygen (normoxia). Venous blood was obtained at baseline in normoxia (0h) following an overnight fast and after 8h and 15h exposure to each respective inspirate. CSF samples were obtained after 18h. Following blood sampling, participants consumed a liquid meal standardised for body mass. Plasma PYY-like immunoreactivity (PYY₃₋₃₆ + PYY₁₋₃₆) and PP were measured via radioimmunoassay. Serum and CSF *N*-tert-butyl- α -phenylnitron spin-trapped alkoxyl radicals (PBN-OR) were determined via electron paramagnetic resonance spectroscopy. Appetite ratings were assessed using a visual analogue scale. Data were

Poster Communications

analysed using a two factor (trial x time) repeated measures analysis of variance and post-hoc Bonferroni-corrected paired samples t-tests.

Results: Compared to normoxia, hunger ratings and the desire to eat decreased during hypoxia whereas satiety, nausea and thirst increased ($P < 0.05$). Hypoxia was associated with a progressive rise in serum and CSF PBN-OR (Table). In contrast, hypoxia did not influence plasma PYY or PP that were shown to decrease with time. Conclusions: These findings fail to support a role for altered PYY and PP as molecular mediators of appetite suppression in response to hypoxia. Further research is warranted to determine if free radicals contribute towards the anorexia associated with AH and to what extent anorectic gut hormones are indeed subject to redox-regulation.

Metabolic responses to hypoxia

Parameter:	Trial:	Blood (0h)	Blood (8h)	Blood (15h)	CSF (18h)
PBN-OR (AU)	Normoxia	5917 ± 1470	5550 ± 1621	6640 ± 1588	1319 ± 233
	Hypoxia	5608 ± 2215	7820 ± 2106*†	9786 ± 2449*†	1840 ± 463†
<i>Effects for Trial, Time, Trial x Time</i>					
PYY (pmol/L)	Normoxia	33.7 ± 15.5	32.4 ± 12.6	28.8 ± 13.1	-
	Hypoxia	31.0 ± 15.4	34.2 ± 16.4	23.8 ± 12.3	-
<i>Effect for Time</i>					
PP (pmol/L)	Normoxia	81.6 ± 51.7	64.7 ± 34.6	27.2 ± 30.0	-
	Hypoxia	76.5 ± 49.7	43.5 ± 14.9	19.6 ± 4.8	-
<i>Effect for Time</i>					

Values are mean ± SD; * different ($P < 0.05$) from 0 h for given Trial; †different ($P < 0.05$) between Trials for given Time;

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PC052

The effects of insulin on mitral cells activity in the rat main olfactory bulb

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Insulin and leptin, released into the systemic circulation, are known to have important effects on food intake. Most attention has been given to the hypothalamus as a likely target for those actions but there is evidence that receptors for both leptin and insulin are also densely expressed in the olfactory bulb, indicating that these hormones might affect the processing of sensory information linked to food palatability and pursue. The aim of this study was to assess the action of insulin on mitral cell activity using *in vivo* extracellular electrophysiology.

Adult male Sprague Dawley rats were anaesthetised with urethane (7.5ml/ kg, 10% v/v) and positioned in prone position in a stereotaxic frame. Two holes were drilled, one above the main olfactory bulb (MOB) and one above the lateral olfactory tract (LOT), which contains the axons of the mitral cells, the main output neurons of

the MOB. Mitral cells were antidromically identified by stimulation of the LOT and confirmed by their characteristic firing pattern. Above the hole of the recording electrode a tubing containing insulin (30mU, 3 μ l, 10mU/ μ l) was positioned, connected to a syringe for topical administration of insulin. Mitral cells fire phasically with intermittent bursts of action potentials which last >100ms which are separated by intervals of variable length between cells during which there is either decreased activity or complete silence. Moreover, they can be divided into two populations. In the first population, within each burst the instantaneous frequency ranges from 80-100Hz. In the second population, within bursts successive spikes of very short interspike intervals (3-8ms) occur resulting in a bimodal interspike interval distribution and a 'double-band' instantaneous frequency distribution with the first band being 70-100Hz and the second one being 150-250Hz. We call these short-interval spikes 'doublets' and 'triplets'. Out of 17 cells tested, 12 were not affected by insulin but five showed a marked excitatory response, showing an approximately 8-fold increase in the doublets and triplets. Two cells which did not show high frequency events, started firing doublets and triplets as a result of the insulin's application. The increase in firing rate was observed about 10 min after application and lasted approximately 10 min before the firing returned to baseline. This study suggests that insulin applied to the olfactory bulb can affect the firing rate of a proportion of mitral cells. We are now testing whether it is a direct effect on mitral cells and whether systemically applied insulin has the same effects. Our results indicate the ability of the olfactory bulb to respond to changes in insulin blood concentrations driven by physiological fluxes that would typically follow a meal, during obese state or metabolic disease.

This study was supported by a BBSRC scholarship

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PC053

Differential changes in gene expression for FND5 during postnatal development are not associated with any change in plasma irisin concentration in sheep

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Background and Aims: Irisin, a circulatory peptide derived from the FND5 gene, has been proposed as an exercise-induced myokine that influences brown adipose tissue (BAT) thermogenesis [1]. It has also been suggested to be released when stimulated by cold exposure in adult humans [2]. In the new-born sheep there is a transition from nonshivering thermogenesis in BAT to shivering in skeletal muscle [3] to mitigate effective adaptation to the cold exposure of the extra-uterine environment. The aim of the present study was to examine whether this adaptation was

Poster Communications

accompanied with changes in gene expression for FNDC5 within BAT and skeletal muscle or changes in the plasma concentration of irisin.

Methods: Four triplet-bearing mothers were entered into the study and a randomly selected triplet was blood sampled from the jugular vein and then euthanased with an intra-venous injection (Pentobarbital Sodium, 200mg/kg body weight) at 1, 7 or 28 days of age for tissue sampling (n=4 per time point) under Home Office Approval, UK.

Adipose tissue was sampled from the sternal/clavicular region and skeletal muscle from the triceps brachii. Samples were weighed and stored at -80°C. The RNA was extracted and gene expression analysed by qRT-PCR. Relative mRNA expression was calculated using the GeNorm method corrected to the geometric mean of reference genes 18S and RPL19, and expressed in arbitrary units. Plasma irisin was determined after each sample was diluted 1:20 using the Phoenix Pharmaceuticals kit No. EK-067-29. Data are presented as mean \pm SEM and unpaired t-Test used for comparisons with age.

Results: The FCDN5 gene was equally abundant in both BAT and skeletal muscle at 1 day of age. There was then a divergent change in gene expression with age, with a gradual decline in sternal fat, compared with a pronounced increase at 7 days in skeletal muscle followed by a large decrease up to 28 days of age (7 day - 0.8616 ± 0.05305 , (n=4), 28 day 0.2548 ± 0.01412 (n=4); $P < 0.0001$). In contrast there was no change in plasma irisin with age (7 day - 116.5 ± 6.812 (n=4), 28 day - 129.1 ± 11.23 (n=4)).

Conclusions: The increase in FCND5 at one week of age is coincident with the increased recruitment of shivering thermogenesis as the dominant response to acute cold exposure. This was not accompanied with any change in plasma irisin thereby adding to more recent evidence that its release into the circulation is not linked to tissue changes in FCDN5 [3]. The lack of any change in irisin with age further suggests no direct role in thermal adaptation after birth.

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PC054

Teratological evaluation of cyanogenic extracts of cassava (*Manihot esculenta* crantz) in Wister rat

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Teratology is the study of birth defect and the word comes from a Greek word *teras* meaning monsters. It has a rich history of myth, folklore and experimental evidence. With the advent of the germ theory by Weismann in 1880s and the rediscovery of the Mendel's law in 1909, the genetic basis for birth defect was accepted. The first human epidemic of birth defect induced by an environmental agent, linked rubella virus infection to organ defect. Also physical and chemical agent have been studied and known to cause malformation as evident with the thalidomide crises in 1960s. Cassava (*Manihot esculenta crantz*) is an important staple and cheap dietary source of calories for 200-300 million people in the tropics, however its nutritional usefulness is hampered by the presence of toxic cyanogens in its tuber(cortex). Cyanogenic glycosides are phytotoxins which occur in at least 2000 plant species that serves as food in developing society, Cassava been one of these foods containing cyanogenic glycosides. In other to evaluate the potentials of cassava induce teratogenicity and subsequently establish its minimum consumption during gestation fifty female Wister rats were used after determination of conception by cervical smear analysis. Ten per group, comprising of three tests and two control groups (positive and normal control). Cyanide was quantitatively calculated in the cultivars and free cyanide was determined by adapting the method of Smith described by Winton and Winton (1985), which is based on colorimetric reaction of picrate solution while the standard curve was established with potassium cyanide. A dose of 40mg, 80mg, and 160mg/kg of potassium cyanide equivalent of the extract was administered by gastric gavage during the 6th to 17th day of gestation. Litters were examined for gross defect and the parents were sacrifice by cervical dislocation after isofluran anesthesia. The research protocol underwent ethical scrutiny through our Department, Faculty and College ethical committee review process and was finally granted approval. There were multiple still births and resorption sites in the uterus of experimental groups. Retained dead fetus reveals distorted morphology with several visceral perforations. However litters did not show gross malformation. These findings suggest that cassava induce embryotoxicity occur during early development but litters who escape the toxic effect during early embryogenesis develop resistance probably due to adaptation and genetic variability.

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PC055

The continuum of adiposity and its effect on skeletal muscle size, structure and function in untrained young versus old males

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Background: In young females, obesity acts as a loading stimulus thus incrementing both skeletal muscle strength (1) and size (2). Nonetheless, the rate of change on skeletal muscle strength and size are in fact exacerbated in obese/highly adipose females (2). Studies show a gender difference in skeletal muscle volume responsiveness to BMI-induced loading (3). Therefore, we hypothesised that obesity would exacerbate the deleterious impact of ageing on skeletal muscle specific force in a male population.

Method: Thirty-four untrained healthy males categorised by age into young (Y) (mean \pm SD: 25.3 \pm 9.6 yrs) versus old (O) (68.7 \pm 7.2 yrs) were recruited to take part in this study. Body fat was assessed using dual-emission X-ray absorptiometry. Plantar flexion maximum voluntary isometric contraction (MVC) torque was assessed in the dominant limb using an isokinetic dynamometer at 0 deg (neutral ankle position) plantar flexion. MVC joint torque was corrected for voluntary muscle activation level (assessed using the interpolated twitch technique) and antagonist muscle co-activation (assessed via surface EMG) and classified as net MVC (nMVC). Gastrocnemius medialis (GM) muscle volume (V) was measured using B-mode ultrasonography and this allowed GM intrinsic strength (nMVC/V) and specific force (SF) to be calculated.

Results: BMI was positively associated with nMVC (Y $r=0.688$; $p=0.005$; O $r=0.538$; $p=0.021$) and V (Y $r=0.762$; $p=0.002$; O $r=0.471$; $p=0.049$). BMI correlation against GM SF was positive in the younger cohort (Y $r=0.773$; $p=0.018$) but not the old (O $r=0.14$; $p>0.05$), so that the difference between the two slopes was statistically significant (Y vs. O being 0.879 N/cm²/BMI vs. 0.145 N/cm²/BMI; student t-statistic 2.05, $p<0.05$). Total body adiposity elicited similar positive associations with nMVC

(Y $r=0.520$; $p=0.047$; O $r=0.585$; $p=0.011$) and V (Y $r=0.708$; $p=0.005$; O $r=0.548$; $p=0.019$). Interestingly the rate of ageing, implied through regression slopes, in terms of both intrinsic strength (-0.639 Ncm/cm³/year $p=0.013$) and GM SF (-0.373 N/cm²/year; $p=0.013$) was significantly steeper in the obese (i.e. high BMI). Similarly, the rate of ageing in terms of both GM V (-1.577 cm³/year; $p<0.05$) and GM SF (-0.183 N/cm²/year; $p<0.05$) was found to be faster in the highly adipose.

Conclusion: This study demonstrates that whilst high BMI (in spite of high adiposity) has a positive loading effect on absolute torque and muscle volume in young males, in older males, presence of obesity is detrimental to skeletal muscle function. Specifically the implied rate of ageing, at the fascicular level, suggests that the endocrine effects of combined adiposity and ageing may be additive. This effect would override the mechanical loading of chronic high loads and hence impede the physiological pathways responsible for muscle function optimization.

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PC056

The objective measurement of feeding behaviour and associations with obesity, genetic variation and health

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Experimentally increasing the rate of food consumption can disassociate satiety from the amount of food ingested, potentially leading to overeating. The Mandometer is a portable weighing scale connected to a small computer that can generate data recording detailed patterns of eating behaviour. The user puts a measured portion of food on the scale and the computer records and displays, in real time, the weight loss from the plate as the user eats: time zero on the graph effectively displays total portion size. This approach to parameterising an otherwise difficult to measure lifestyle factor has not been applied to the general population, to date only being used in therapeutic settings. Here we used the Mandometer to measure natural variation in eating behaviour in a random sample from an existing

cohort study, The Avon Longitudinal Study of Parents and Children. In 91 samples assessed over three meals, those with complete data showed an average meal weight for normal weight category was estimated to be 275g (95% CI 251-298g). On average, normal weight participants consumed food at a rate of 0.24g per second (95% CI 0.23-0.24). There was evidence that overweight ($p=0.01$) and obese ($p=0.01$) participants had larger meals than normal weight participants on average. Those overweight participants on average had 102g more food than normal weight participants (95% CI 29-175g) and obese participants had 200g more food than normal weight participants (95% CI 41-358g). There was strong evidence that overweight and obese participants ate at a faster rate than normal weight participants. Overweight participants ate 0.2g per second quicker (95% CI 0.19-0.21g/s; $p<0.005$) than normal weight participants, while obese participants ate 0.56g per second faster on average (95% CI 0.52-0.60g/s; $p<0.005$). Furthermore, there was evidence that carriers of different genotypes at a confirmed fat mass and obesity related genetic locus (FTO) thought to act through patterns of consumption, showed different Mandometer results. Those carrying the T/T genotype on average had 130g less food than those carrying the rare A/A homozygote genotype (95% CI 57-204g) with a trend observed for the heterozygote state (45g less on average 95% CI (19-111g)). There was strong evidence that carriers of the common allele at rs9939609 (T) ate at a slower rate than normal weight participants. T/T participants ate 0.3g per second slower (95% CI 0.28-0.26g/s; $p<0.001$) than those with an A/A genotype. Of note, these patterns were correlated with pertinent health factors such as blood pressure measured at approximately the same age (~22 years). Taken together, this work highlights the ability of the Mandometer to be used as an effective measure of eating behaviour at the population level.

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PC057

Oestrogen receptor expression and function in GLP-1 secreting GLUTag L-cells

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Glucagon-like peptide-1 (7-36) amide or GLP-1 is an incretin hormone released postprandially from intestinal endocrine L-cells¹. GLP-1 has multiple metabolic regulatory functions including glucose-dependent potentiation of insulin secretion, inhibition of gastrointestinal secretions, reduced motility and delayed gastric emptying thereby reducing caloric intake, enhancing satiety and aiding weight loss². Oestrogen is also an important hormone regulating metabolic homeostasis in both males and females, and while most of its actions are attributed to nuclear receptors ER α and ER β , rapid non-genomic signalling may also be mediated via the G protein-coupled oestrogen receptor (GPER)³. It has been reported that GLP-1

levels increase during the third trimester of pregnancy but also that fasting GLP-1 is reduced post-menopause suggesting potential regulation of circulating GLP-1 levels by oestrogen and other female sex hormones. It is known that oestrogen receptors are expressed in intestinal epithelial cells, but their expression and potential role in intestinal endocrine cells has not been established. Aims: This study aims to identify the expression and role of oestrogen receptors and their agonists on L-cell function and secretion of GLP-1 using the established GLUTag cell line. Methods: The expression of oestrogen receptors in GLUTag cells was assessed using PCR and immunocytochemistry. The effects of nuclear and membrane oestrogen receptor agonists on changes in intracellular calcium was examined in Fura-2 loaded GLUTag cells. GLP-1 release was assessed over 2h using GLUTag cells seeded on matrigel coated 24-well plates, with measurement of GLP-1 in supernatant using the Total GLP-1 ELISA kit, Millipore. Results: ER alpha and beta and GPER are expressed in GLUTag L-cells, suggesting a potential role for oestrogen in function of GLP-1 secreting cells. In the presence of glucose, oestrogen did not appear to have any significant effect on intracellular calcium, however glucose-dependent (10 mM) GLP-1 was enhanced 1.6 – 1.8- fold ($P < 0.05$ - 0.01) by oestrogen. GPER agonist G1 had little effect on GLP-1 secretion. Interestingly, ER α agonist PPT and ER β agonist WAY200070 had inverse concentration-dependent inhibitory effects on GLP-1 release indicating a potential inhibitory role at ER alpha activating concentrations. Conclusion: Oestrogen receptors may play a role in regulating GLP-1 secretion, with native oestrogen having a stimulatory effect on GLP-1 secretion, suggesting that oestrogen receptor activation may increase endogenous GLP-1 as a means to regulating satiety. However, the role of specific oestrogen receptors in L-cell function and GLP-1 secretion require further investigation.

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PC058

Characterisation of the role of adiponectin in rodent models of obesity and type 2 diabetes with the development of co-morbid pain

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Adipose tissue derived cytokines, such as the anti-inflammatory adiponectin play a significant role in a variety of disease conditions. Adiponectin is reduced with obesity and in type 2 diabetes patients; both conditions associated with development of co-morbid pain. This study set out to characterise expression of adiponectin in spinal cord in rodent models of genetic obesity (Zucker rats), experimentally-induced obesity (high fat diet (HFD) fed rats), and type 2 diabetes, and to determine if changes are associated with alterations in nociceptive pain.

Responses to thermal and mechanical stimulation of the hindpaw were assessed in adult male Zucker fatty rats and lean littermates (n=6/group), adult male Wistar rats fed a high fat (22%) diet for 16 weeks and injected intraperitoneally (i.p) with either low dose of streptozotocin (STZ; 30 mg/kg; (n=6), a model of type 2 diabetes (T2D rats), or vehicle (HFD rats; n=6), and control rats fed a normal diet and injected with vehicle citrate buffer (i.p; n = 6). Animals were euthanased by schedule 1 killing and spinal cord and plasma collected. Blood glucose, insulin and total cellular cholesterol were measured, and expression of adiponectin mRNA analysed in spinal cord using real-time PCR (n=6 per group). Data were analysed using an ANOVA with post-hoc Tukey's test.

Obese Zucker rats were significantly heavier than lean controls ($551 \pm 35\text{g}$ vs. $377 \pm 27\text{g}$; $P < 0.01$), as were HFD rats ($502 \pm 12\text{g}$ vs. $444 \pm 7\text{g}$ for controls; $P < 0.01$). Both obese Zucker and HFD rats had elevated plasma cholesterol and insulin levels (all $P < 0.01$ vs. controls), but were normoglycaemic. After an initial weight gain, T2D rats had significant weight loss (410 ± 12 vs. $473 \pm 4\text{g}$ for controls; $P < 0.01$), and had elevated blood glucose and cholesterol levels ($P < 0.05$ and $P < 0.01$ vs. controls); insulin levels were unchanged. T2D rats displayed significant thermal and mechanical hyperalgesia ($P < 0.01$ vs. controls); nociceptive responses were unchanged in Obese Zucker and HFD rats. Real-time PCR analysis revealed a significant down-regulation in spinal adiponectin in T2D rats (8 fold decrease; $P < 0.05$ vs. control rats), and in obese Zucker rats (2.4 fold decrease; $P < 0.05$ vs. lean rats, but not in HFD rats).

The increased pain sensitivity and altered adipokine expression profile in rats with type 2 diabetes fits the hypothesis that changes in key adipokines, at the spinal level may underlie pain with diabetes, although further studies are necessary to confirm a causative role.

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PC059

DLK1/PREF1 regulates nutrient metabolism and protects from steatosis by elevating pituitary growth hormone secretion

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The Delta-like homologue 1 (Dlk1)/Preadipocyte factor 1 (Pref1) gene encodes a signalling protein known to influence pathways of cellular proliferation and differentiation¹. Dlk1 is an imprinted gene, and we have previously shown that its dosage is critically regulated during embryonic development such that only 3-fold overexpression causes prenatal death². Intriguingly, DLK1 levels are naturally elevated at several crucial life-history periods in both humans and rodents; in perinatal life and in maternal tissues and serum in the later stages of pregnancy³. Since the expression dosage of Dlk1 is tightly controlled, we wished to discover - what is the normal biological function of elevated DLK1? In addition to its dosage regulation, Dlk1 has a complex pattern of splice- and tissue-specific regulation. Past studies addressing the effects of DLK1 overexpression have not considered its restricted normal expression pattern, or the contribution of all splice forms, leading to some confusion about the in-vivo role for this protein.

In order to address the function of high DLK1 in normal development we utilised a transgenic model of Dlk1 overexpression from endogenous control elements. We confirmed that the transgenic animals have elevated DLK1 in tissues that normally express the protein, and that the correct splicing pattern is maintained (Figure 1). By comparing the phenotype of wild-type mice with Dlk1-transgenic animals in a variety of genetic/dietary backgrounds, we have demonstrated:

1) In contrast to the current dogma that Dlk1/Pref1 is an inhibitor of adipogenesis, overexpressing mice have normal white adipose tissue expansion following both diet- and genetically-induced obesity. Importantly, we instead identify a novel function for this factor in lipid metabolism.

2) We have uncovered its novel mechanism of action - Dlk1-overexpression causes a defect in IGF1 negative feedback regulation and elevated pituitary GH secretion. While Dlk1 has been implicated in GH signalling before, to our knowledge we provide the first demonstration that altered Dlk1 dosage can affect this pathway in a physiologically relevant manner, and we describe a mechanism by which this occurs.

3) The Dlk1-overexpressing transgenic mice are resistant to hepatosteatosis – a finding with important clinical implications.

We concluded that elevated DLK1 dosage causes a repartitioning of energy substrate metabolism, such that fatty acids are more readily utilised as fuel by peripheral tissues, and hepatic lipid production and storage is reduced. Since the dosage of circulating DLK1 is naturally elevated in early life and during pregnancy, we

Poster Communications

believe that our transgenic model mimics endogenous mechanisms of DLK1-mediated GH signalling modulation that are employed during periods of metabolic stress to protect from steatosis and alter fuel utilisation in the whole organism.

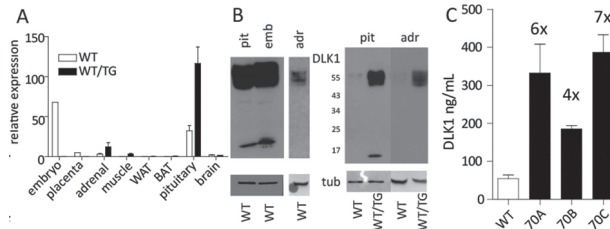


Figure 1. DLK1-TG mice recapitulate the spatial and temporal regulation of the endogenous gene.

A. mRNA levels of DLK1 in prenatal and adult tissues, $n = 4$, mean \pm s.e.m.

B. Expression of DLK1 protein persists in adult endocrine tissues and levels are elevated in the DLK1-TG.

C. Serum DLK1 in 3 independent transgenic lines, 70A, B and C, mean \pm s.e.m.

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PC060

Gender Difference in nutritional status of undergraduate medical students of Bangladesh

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Background: Early adults are considered to be a nutritionally vulnerable segment of the population. Poor nutritional status in early adulthood is an important determinant of health outcomes at a later stage of life.¹ In Bangladesh, approximately 85% of the people intake insufficient food, 76% of rural households are deficient in protein intake and 93%, 88% and 87% of households had deficient intake of

Poster Communications

calcium, Vitamin A and Vitamin C respectively. 2 Medical students pass their early adulthood at medical college. They may have nutritional abnormalities as they can not take proper nutritious food in their dormitories. Moreover, female students get less opportunity to take food from food shops outside the campus. Whether female students suffer from more nutritional abnormality than males is not known. Objectives: To examine the gender difference in nutritional status of undergraduate medical students of Bangladesh.

Method: We conducted this cross-sectional comparative study in the Department of Physiology, Noakhali Medical College, Bangladesh during April–June, 2013. Two hundred one students were selected purposively from Noakhali Medical college of Bangladesh. Permission was taken from authority and consent was taken from the participants. Height (cm) and weight (kg) were measured and BMI was calculated and collected on a data sheet. Data was analyzed by using SPSS (version 16) for windows. Comparison between male and female students was done by chi-square test. P value < .05 was considered significant. Classification of the nutritional status of the students was done according to WHO Global Database on Body Mass Index. Results: The mean \pm SD age of the students was 20.93 ± 1.57 years. Among the participants 84 (40.4%) were male students. The mean \pm SD BMI of the male, female and total students were 22.80 ± 3.69 , 20.71 ± 3.19 and 21.56 ± 3.55 respectively. Among the total students 138 (69%) had normal weight, 37 (18.5%) were underweight, 21 (10.5%) pre-obese and 4 (2.0%) obese. Underweight was significantly more prevalent in female students (23.7%) than male (11.0%) and pre-obesity and obesity were significantly more prevalent in male than female students ($p = .008$). Conclusion: Almost one-third medical students suffer from nutritional abnormality. 18.5% suffer from underweight and 12.5% from overweight. Female students suffer significantly more from underweight and male students suffer significantly more from overweight.

Nutritional status of the students (n=201)

	Nutritional status of the students			P value
	Male (n=82) n (%)	Female (n=119) n (%)	Total (n=201) n (%)	
Normal	56(68.3)	82(69.5)	138(69.0)	.008
Underweight	9(11.0)	28(23.7)	37(18.5)	
Pre-obese	14(17.1)	7(5.9%)	21(10.5)	
Obese	3(3.7)	1(0.8)	4(2.0)	

P value derived from chi-square test

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PC061

Genistein promotes a gene expression profile characteristic of brown or beige, rather than white, adipocytes and increases Sirt1 expression in mouse NIH3T3-L1 cells

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Various possible benefits have been attributed to the dietary isoflavone genistein. In common with resveratrol, which may act via sirtuin 1 (Sirt1), these benefits include protection from diet-induced obesity. We thus aimed to determine if genistein affected adipose tissue physiology and/or Sirt1 expression using a cell culture model.

NIH3T3-L1 cells were differentiated into adipocytes (from 48 h post-confluence, taken as day 0) in the presence or absence of genistein. Gene expression was measured by RT-qPCR, and data expressed as mean \pm SEM for n=3-6 relative to control. Mitochondrial oxygen consumption was measured using the Seahorse Bioscience XF system and expressed as mean \pm SEM for n=10. Statistical analysis of data was by one-way ANOVA followed by Dunnett's test for RT-qPCR data and by Student's t-test for data on mitochondrial oxygen consumption.

A low concentration of genistein (10 μ M) and/or shorter exposure (days 0-12) promoted differentiation to white adipocytes, indicated by large fat droplets and increased expression of adipocyte marker genes *Acaca* (acetyl Co-A carboxylase- α) (1 ± 0.092 ; 1.88 ± 0.007 ; $P < 0.002$), *Fasn* (fatty acid synthase) (1 ± 0.057 ; 2.2019 ± 0.135 ; $P < 0.0001$), *Fabp4* (fatty acid binding protein 4) (1 ± 0.056 ; 2.56 ± 0.37 ; $P < 0.007$) *Lipe* (hormone sensitive lipase) (1 ± 0.043 ; 2.43 ± 0.212 ; $P < 0.0012$), *Retn* (resistin) (1 ± 0.074 ; 2.36 ± 0.34 ; $P < 0.008$), *Rarres2* (chemerin) (1 ± 0.022 ; 2.56 ± 0.27 ; $P < 0.002$)). However, at higher concentrations of genistein (50-100 μ M) and/or after longer exposure (days 0-12) cells had smaller fat droplets and lower expression of these genes, coupled with increased expression of *Sirt1* (1 ± 0.037 ; 2.113 ± 0.065 ; $P < 0.00004$) and of genes characteristic of brown adipocytes (*Ucp1* (uncoupling protein 1) (1 ± 0.10 ; 1.63 ± 0.17 ; $P < 0.009$), *Tnfrsf9* (tumor necrosis factor receptor superfamily member 9) or (CD-137) (1 ± 0.06 ; 1.37 ± 0.021 ; $P < 0.0021$), and *Cebpb* (CCAAT/enhancer binding protein- β) (1 ± 0.056 ; 2.78 ± 0.11 ; $P < 0.00007$)). In addition, basal and proportion of uncoupled mitochondrial oxygen consumption were higher in cells treated with genistein than in control cells (253 ± 11 versus 305 ± 7 pmol/min/mg; 0.54 ± 0.04 versus 0.87 ± 0.06 , respectively; $P < 0.001$) consistent with a switch in metabolic phenotype towards brown or beige adipocytes.

Dietary genistein may thus protect against obesity by encouraging the development of brown or beige, rather than white, adipose tissue, possibly through a mechanism involving Sirt1.

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PC062

Behavioral and electrophysiological improvements after combined treatment with melatonin and mesenchymal stem cells in injured sciatic nerve in rats

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Mesenchymal stem cells have some ameliorating effects in peripheral nerve injury. Melatonin also proved to be a protective agent against cellular oxidative damage. The effect of transplantation of mesenchymal stem cells in peripheral nerve injury combined with melatonin have not been tested before. The aim of our study was to evaluate this combined effect on the improvement of the injured sciatic nerve in rats.

Fifty female albino rats were divided equally into 5 groups: shame control-intact peripheral nerve, control injured sciatic nerve group, injured sciatic nerve melatonin treated group (total 10mg/kg intra peritoneal divided into 2.5 mg/kg at 20 min pre, at the time, at 60 min and at 120 min post injury), injured sciatic nerve mesenchymal stem cells treated group (3x 10⁵ cells/rat, diluted in PBS and transplanted intralesionally using insulin syringe directly after injury) and injured sciatic nerve combined melatonin and stem cells treated group with same previous doses. Behavioral assessment using the Walking Track analysis were performed in all groups once before injury and at fourth and eighth weeks after injury. Nerve conduction and EMG, using the Biopack, MP150 system were done at 8th week post injury. The measurement of total antioxidant capacity and malonaldehyde oxidase enzyme in serum were done 48 hours post injury.

Mesenchymal stem cells were isolated from the human umbilical cord blood using the Ficoll-Hypaque density gradient centrifugation, then culture of mononuclear cells and selection by CD105+veCD34-veCD45-ve magnetic separation method using MACS separator.

Injury to the sciatic nerve was done using the standardized crush injury method under general anesthesia by pentobarbital sodium (100 mg/kg). Complete post operative care was performed to all groups. The study was approved by the institutional ethical committee and carried out in accordance with the current guidelines for the care of lab animals.

Treated groups showed significant improvement in all tested parameters compared to the injured non treated groups. The combined treatment group showed

the best results of improvement in the behavioral and electrophysiological assessment.

Conclusion: Treatment with combination of mesenchymal stem cells and melatonin gives better improvement in the sciatic nerve injury than using each one of them separately.

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PC063

oboe – A novel mouse model of mitochondrial disease

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In 2011, *WARS2* was identified within a locus associated with waist-hip-ratio (WHR) in a human GWAS study (1). WHR is a measure of body fat distribution. *WARS2* expression was shown to be significantly reduced in both the subcutaneous and visceral adipose tissue of obese humans compared with lean humans (2). *WARS2* encodes mitochondrial tryptophanyl tRNA synthetase. Mitochondrial aminoacyl tRNA synthetases (mtRS) are essential to the generation of complexes within the oxidative phosphorylation pathway (OXPHOS). Mutations within several mtRSs encoding genes can cause a range of mitochondrial disorders in humans with highly variable clinical presentations (3). However there is an unexplained relationship between the disease affected tissue and the affected mtRS. To date, no patient with mitochondrial disease has been documented with a *WARS2* mutation. Here I briefly describe a novel mouse model 'oboe' which provides a useful vehicle to investigate the tissue specific relationship between *WARS2* and adipose tissue.

oboe was identified from 'The Harwell Ageing Screen' as having significantly reduced total body mass. Affected *oboe* mice are significantly lighter than littermates from 7 months of age. Body composition analysis at 15 months indicated that the reduction in body mass is representative of significantly reduced fat mass. Affected *oboe* mice had a mean total fat mass of 5.7g compared with 21.2g in control mice. SNP analysis mapped the lean phenotype to a 73Mb region of chromosome 3 within which a novel ENU-induced mutation in *Wars2* was identified. The ENU-induced mutation encodes an amino acid change and induces a novel mis-splicing event where there is complete excision of exon 3. RT-PCR analysis showed that there is less than 40% full-length *Wars2* mRNA in *Wars2*^{oboe/oboe} mouse embryonic fibroblasts (MEFs) compared to *Wars2*^{+/+} MEFs. Mitochondrial OXPHOS

function was characterized and unexpectedly both basal oxygen consumption and maximal respiratory capacity were significantly increased in *Wars2^{oboe/oboe}* MEFs compared to *Wars2^{+/+}* MEFs. Total mitochondrial mass was also approximately 40% increased in *Wars2^{oboe/oboe}* MEFs. The relative expression of *Pgc-1 α* , the ‘master regulator’ of mitochondrial biogenesis was over 70% increased in *Wars2^{oboe/oboe}* MEFs compared with *Wars2^{+/+}* MEFs.

We hypothesise that the reduction in full-length *Wars2* transcript in *oboe* mice inhibits mitochondrial translation and by extension OXPHOS function. This reduced OXPHOS function triggers a compensatory mechanism through activation of the cellular energy sensor *AMPK* to inhibit anabolic and up-regulate catabolic pathways including: mitochondrial biogenesis, glycolysis, and fatty acid oxidation. Increased catabolic metabolism may explain the lean phenotype observed in *oboe* mice. The overall aim of this ongoing project is to investigate whether the *Wars2^{oboe}* ENU-induced mutation has an adipose tissue specific effect.

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PC064

Obesity in women can result in uterine dysfunction due to dysregulation of hERG K⁺ channel activity

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Obese women tend to have delayed and/or weak labour that can result in caesarean section. The mechanisms regulating uterine smooth muscle contraction are poorly understood and this limits the ability to clinically control events when these mechanisms malfunction. A plateau phase is particularly prominent in the AP of human uterine smooth muscle and the duration of the plateau plays a major role in determining the duration of contraction. The ionic conductances responsible for determining the amplitude and duration, and the rapid repolarization of the plateau in human myometrium are unknown. A plateau is also a prominent feature of the AP in the heart and hERG1 K⁺ channels contribute substantially to

repolarization. ERG has been identified in a range of smooth muscle tissues, including mouse myometrium. In the present study, we obtained human myometrium following caesarean delivery, having obtained informed written consent from the women. We recorded membrane potential and contraction simultaneously in strips of myometrium, ion currents were recorded in smooth muscle cells isolated from the same tissue samples and hERG protein levels were determined using Western blotting. The hERG blockers dofetilide and E-4031 prolonged the duration of the plateau phase of the AP and contraction, from 0.9 ± 0.2 min to 2.8 ± 0.2 min (a 2.9 fold increase, $n=10$, $p<0.001$). The hERG activator ICA-195574 reduced contraction duration to 53% ($n=7$). The hERG current in acutely isolated myometrial cells had a maximum amplitude of 3.6 ± 0.4 pA/pF and was blocked by dofetilide and E-4031. In tissues obtained from women in established labour, dofetilide increased AP duration from 2.8 ± 0.1 to 3.6 ± 0.1 ($p=0.008$, $n=7$), an increase of only 1.3 fold, and the maximum hERG current was reduced to 1.3 ± 0.4 pA/pF. Western blotting revealed that the levels of the α pore-forming hERG subunit remained unchanged before and during labour. However, levels of the β auxiliary subunit, which suppresses the hERG current, were increased 2.4 fold during labour ($p<0.0001$, $n=12$ in each group). As BMI increased, the effectiveness of dofetilide in prolonging AP duration was enhanced before ($r^2=0.89$) and during ($r^2=0.68$) labour, suggesting enhanced hERG expression/activity. Further investigation identified a striking reduction in β subunit levels with increasing BMI ($r^2=0.70$) in tissue from women in labour. Thus, hERG channels suppressed AP duration and contraction amplitude and duration before labour, facilitating quiescence. Then during labour, levels of the inhibitory β subunit are doubled, resulting in suppressed hERG activity, an increase in AP duration and larger contractions, facilitating vaginal delivery. In obesity this system fails, associated with maintained levels of the inhibitory β subunit, and likely contributes to the poor labour and increased incidence of caesarean delivery in many obese women.

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PC065

Consumption of high sucrose and/or high salt diet alters sperm function in male Sprague-Dawley rats

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Increased prevalence of obesity and impaired glycemic control is a major challenge for modern western society. Clinical studies have indicated that obesity is

an independent predictor of cardiovascular risk. Metabolic syndrome, a cluster of hyperglycemia, hypertension, excess body fat and abnormal cholesterol, is associated with impaired vascular functions such as endothelial dysfunction and arterial stiffness. The mechanism leading to these vascular abnormalities is not well understood. In our current study, we fed male C57BL/6J mice with a high fat/high sucrose (HFHS) diet for 6 months with or without treatment with the arginase inhibitor ABH (2-(S)-amino-6-boronohexanoic acid, 10 mg/kg/day) in drinking water. Elevated activity of arginase (ARG), an enzyme implicated in many cardiovascular diseases, can compete with nitric oxide (NO) synthase for their common substrate, L-arginine. This leads to reduced bioavailability on NO and endothelial dysfunction. Increased arginase activity also provides more ornithine for synthesis of polyamines via ornithine decarboxylase (ODC) and proline/collagen via ornithine aminotransferase (OAT), leading to vascular cell proliferation and collagen formation, respectively. We hypothesized that elevated arginase activity is involved in vascular dysfunction and arterial fibrosis/stiffness associated with obesity related type 2-diabetes and that limiting its activity can prevent these pathologies. In our study, HFHS significantly increases body weight and fasting glucose levels, starting and progressing after 8 weeks of diet. We observed increased aortic and plasma arginase activity (blood collected by cardiac puncture after Ketamine/Xylazine anesthesia), increased plasma lipid peroxidase activity (measure of systemic oxidative stress), impaired endothelial function (relaxation response to acetylcholine in isolated aortic rings) and arterial stiffness (aortic pulse wave velocity measured under 1.5%-isoflurane anesthesia using ultrasound) in HFHS mice but not in HFHS mice treated with ABH. Aortic perivascular collagen deposition was significantly higher in HFHS mice compared to normal diet and HFHS+ABH mice. Furthermore, marked increase in circulating blood monocyte (flow cytometry) and macrophage (CD68+) infiltration into the aortic walls was observed. HFHS mice treated with ABH did not show these effects. In conclusion, elevated arginase activity is involved with the pathophysiology of obesity induced type 2 diabetes. Treatment with arginase inhibitor ABH ameliorates HFHS induced endothelial dysfunction, arterial stiffening, oxidative stress, and aortic macrophage infiltration.

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PC066

Protective potential of chromatographic fractions of *Spondias mombin* L. (Anacardiaceae) on sodium arsenite exposed Wistar rats

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Arsenic is present in the environment as a naturally occurring substance or due to contamination from human activity. Arsenic may also be found in many foods, including grains, fruits, and vegetables, where it is present due to absorption from the soil and water. This study focused on inorganic arsenic, which often occurs in excess in the drinking water of millions of people worldwide, and has been previously shown to be a human carcinogen. Herbal medicinal practice is now very common worldwide and in this study, safety and protective potential of the chromatographic fractions of *Spondias mombin* (SM) was evaluated by studying the haematological and serum biochemical variations accompanying oral administration of chromatographic fractions of *Spondias mombin* to sodium arsenic-exposed wistar rats. Thirty five physically healthy male albino rats (125 – 228 g) were used, which were divided into 7 groups (groups A to G) of 5 rats each. Group A was treated with 0.1ml Dimethylsulphoxide (DMSO), B (0.1ml of Distilled water), C (Sodium arsenite (SA) 2.5mg/kg body weight), D (Ethylacetate fraction), E (Ethylacetate fraction for 7 days and SA on the 7th day), F (Methanolic fraction for 7days and SA on the 7th day) and G (Methanolic fraction alone for 7 days). This study showed that the oral administration of the chromatographic fractions of *Spondias mombin* significantly increased ($p < 0.05$) the PCV, HB, MCV and MCH values for group G when compared to the control group B. In addition, the Lymphocytes and Neutrophil count for group D was also significantly increased ($P < 0.05$). The changes in the mean values of the liver markers ; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamic transpeptidase (GGT), total protein and albumin for the fraction treated groups were reduced significantly ($P < 0.05$) when compared to the control group B except for group G that exhibited significant increase ($P < 0.05$) in the serum ALT and albumin level. The fraction treated group F, in addition to arsenic treated group caused significant increase ($P < 0.05$) in the blood urea nitrogen level while all the fraction treated groups caused a significant reduction ($P < 0.05$) in the blood glucose concentration. Thus, against the hepatotoxic and kidney toxicity induced by sodium arsenite, methanol and ethyl acetate fractions of *Spondias mombin*, exhibited hepatoprotective, nephroprotective, antianaemic, anti- infection and hypoglycemic properties.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC067

Cardiovascular implications of arginase upregulation in animal model of obesity related diabetes

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Increased prevalence of obesity and impaired glycemic control is a major challenge in the western society. Clinical studies have indicated that obesity is an independent predictor of cardiovascular risk. Metabolic syndrome, a cluster of hyperglycemia, hypertension, excess body fat and abnormal cholesterol, is associated with impaired vascular functions such as endothelial dysfunction and arterial stiffness. The mechanism leading to these vascular abnormalities is not well understood. In our current study, we fed C57BL/6J mice with a high fat/high sucrose (HFHS) diet for 6 months with or without treatment with the arginase inhibitor ABH (2-(S)-amino-6-boronohexanoic acid, 10 mg/kg/day) in drinking water. Elevated activity of arginase (ARG), an enzyme implicated in many cardiovascular diseases, can compete with nitric oxide (NO) synthase for their common substrate, L-arginine. This leads to reduced bioavailability on NO and endothelial dysfunction. Increased arginase activity also provides more ornithine for synthesis of polyamines via ornithine decarboxylase (ODC) and proline/collagen via ornithine aminotransferase (OAT), leading to vascular cell proliferation and collagen formation, respectively. We hypothesized that elevated arginase activity is involved in vascular dysfunction and arterial fibrosis/stiffness associated with obesity related type 2-diabetes and that limiting its activity can prevent these pathologies. In our study, HFHS significantly increases body weight and fasting glucose levels, starting and progressing after 8 weeks of diet. We observed increased aortic and plasma arginase activity, increased plasma lipid peroxidase activity (measure of systemic oxidative stress), impaired endothelial function (relaxation response to acetylcholine in isolated aortic rings) and arterial stiffness (aortic pulse wave velocity) in HFHS diet mice but not in HFHS mice treated with ABH. Aortic perivascular collagen deposition was significantly higher in HFHS mice compared to normal diet and HFHS+ABH mice. Furthermore, marked increase in circulating blood monocyte (flow cytometry) and macrophage (CD68+) infiltration into the aortic walls was observed. HFHS mice treated with ABH did not show these effects. In conclusion, elevated arginase activity is involved with the pathophysiology of obesity induced type 2 diabetes. Treatment with arginase inhibitor ABH ameliorates HFHS induced endothelial dysfunction, arterial stiffening, oxidative stress, and aortic macrophage infiltration.

Poster Communications

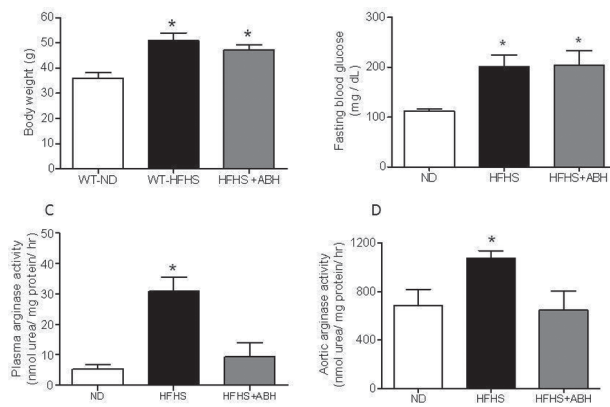


Fig 1. C57BL/6 mice subjected to high fat/high sucrose (HFHS) for 6 months increases: A. body weight, B. fasting blood glucose C. plasma arginase activity D. aortic arginase activity. Treatment with arginase inhibitor ABH has no effect on HFHS induced increase in body weight and hyperglycemia. Values are expressed as mean \pm SEM, n = 6, *P < 0.05 vs. ND (normal diet). One way ANOVA with a Tukey post test.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC068

Crosstalk between leptin and interleukin-6 in the pathophysiology of Irritable Bowel Syndrome

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Background: Irritable Bowel Syndrome (IBS) is a chronic condition characterised by bouts of cramping, abdominal pain, constipation and/or diarrhoea and it afflicts up to 10-20% of the population. Symptom flares are exacerbated by factors such as stress or food intake and support is now growing for a role for immune activation in symptom manifestation. Indeed, the pro-inflammatory cytokine, interleukin (IL)-6, is elevated in IBS plasma and can activate enteric neurons leading to changes in gastrointestinal secretion. However, other plasma constituents such as the satiety hormone, leptin are also altered in IBS plasma. This may reflect a protective role of leptin in IBS or it may be important in regulating the function of IL-6. Indeed, leptin can also activate enteric neurons and alter gut function. With similar signalling pathways, these studies aim to investigate potential crosstalk between leptin and IL-6 in the pathophysiology of IBS.

Methods: Whole mount preparations of submucosal and myenteric neurons were prepared from adult male Sprague Dawley rats and were loaded with the ratio-metric calcium indicator, Fura 2AM (7 μ M). Real-time calcium imaging experiments were conducted using a standard epifluorescence imager. Neurons were also fixed, permeabilized and incubated with antibodies for immunofluorescence staining.

Results: Leptin induced a significant increase in intracellular calcium in submucosal (0.146 \pm 0.02, n=34) neurons but there was no effect on myenteric neurons. Leptin attenuated IBS plasma-evoked activation in both myenteric neurons and submucosal neurons (P<0.001). Recombinant IL-6 caused a significant increase in calcium which was reduced in the presence of leptin (P<0.01, n=38) in myenteric neurons, but leptin did not significantly alter the response in submucosal neurons. In submucosal neurons, leptin-evoked responses were unaffected by the MAPK inhibitor, PD98059 but potentiated the response to IL-6 and leptin (P<0.001). In contrast, the STAT3 inhibitor, WP1006 attenuated the response to leptin (P<0.001) but also increased the response to IL-6 and leptin (P<0.05). Immunofluorescent expression of both leptin and leptin receptors co-localised with IL-6 receptors in both submucosal (64%) and myenteric (39%) neurons.

Conclusions: These data provide tangible evidence of interaction between leptin and IL-6 in colonic enteric neurons, with leptin having a moderating effect on the stimulatory actions of IL-6 in myenteric but not in submucosal neurons. These data suggest a potentially protective effect of leptin in functional bowel disorders.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC069

A natural solution for Obesity – Devil's Claw attenuates food intake via ghrelin receptor modulation

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Ghrelin is a stomach-derived peptide that acts as an orexigenic hormone in the hypothalamus stimulating food intake through the growth hormone secretagogue receptor (GHS-R1a). Dysregulated ghrelin signalling may contribute to the development of metabolic disorders such as obesity. Current pharmacologic anti-obesity treatments lack efficacy and have shown severe side effects, highlighting the urgent need for novel strategies contributing to the maintenance of a healthy weight. Natural products are receiving special consideration as sources of bioactives with potential beneficial health effects which may be safer and more attrac-

tive for consumers than synthetic therapeutics. One example is the plant extract derived from the dried tuberous roots of *Harpagophytum procumbens* which has been used traditionally as herbal medicine for a variety of conditions, and currently is mainly used as an anti-inflammatory agent and as an analgesic. In addition, it has some folkloric precedent as a modulator of appetite. This study aims to investigate the effect of *H. procumbens* on GHS-R1a receptor modulation *in vitro* and analyse its effects on food intake *in vivo*. GHS-R1a receptor activating potential of *H. procumbens* soluble extract was analysed by calcium mobilization and receptor internalization assays in human embryonic kidney cells (Hek) stably expressing the GHS-R1a receptor. Furthermore, cumulative food intake was investigated in male C57BL/6 mice following intraperitoneal (IP) administration of the soluble *H. procumbens* extract. Exposure to *H. procumbens* extract demonstrated a significant increased cellular calcium influx but did not induce subsequent GHS-R1a receptor internalization, which is characteristic of full receptor activation. A significant anorexigenic effect was observed in male C57BL/6 mice following peripheral administration of *H. procumbens* extract. We conclude that *H. procumbens* root extract is a potential novel source of potent anti-obesity bioactives. These results reinforce the promising potential of natural bioactives to be developed into functional foods with weight-loss and weight maintenance benefits.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC070

Investigation of ghrelin and serotonin 2C receptor crosstalk on food intake

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Globally the incidence of obesity has reached epidemic proportions and still continues to rise. In fact, obesity is now recognized as the fifth leading cause of global death and arguably the number one health concern within developed countries ahead of heart disease and cancer. The negative effects of obesity have now been considered to outweigh that of smoking and alcohol abuse. In addition, obesity associated morbidities such as cardiovascular disease and type II diabetes are becoming an increasing burden for economy and healthcare. Obesity is now viewed as one of the most profound failures in pharmacotherapy, in part due to poor safety and efficacy reports of previous anti-obesity drugs as well as the lack of targets for new drug discoveries highlighting an unmet need for novel strategies. The 5-HT_{2C} receptor is becoming a major target for the development of new anti-obesity therapeutics as this receptor is implicated in satiety stimulation, highlighted by the recent approval of Lorcaserin, a 5-HT_{2C} agonist. Interestingly,

Poster Communications

recent studies have demonstrated a functional interaction between the 5-HT_{2C} receptor and the receptor of the “hunger” hormone ghrelin, also known as the growth hormone secretagogue receptor (GHS-R1a). This suggests a potential novel mechanism of enhancing appetite regulation through functional cross talk of these two receptors. This study aims to investigate if there is a synergetic link between the appetite suppressing effects of the 5-HT_{2C} receptor and GHS-R1a. In vitro analysis showed an attenuating effect of 5-HT_{2C} receptor signalling on GHS-R1a-mediated intracellular calcium mobilization. Furthermore, examining cumulative food intake in C57Bl/6 mice it was established that there was a potentiation of ghrelin’s orexigenic effect following intraperitoneal (IP) administration of ghrelin in combination with the 5-HT_{2C} antagonist SB242084, when compared to administration of ghrelin or SB242084 on their own. Following these results further studies will now be carried out to determine if there is an interaction between the appetite suppressing effects of the recently marketed 5-HT_{2C} receptor specific agonist lorcaserin and the ghrelinergic system. The existence of a GHS-R1a/5-HT_{2C} receptor dimer will enhance the pharmacological diversity and offer a novel pharmacological target for a range of feeding behaviours and functions relating to the ghrelinergic system including homeostatic appetite signalling and hedonic regulation of food intake behaviours.

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A

Abdelmoemen, E.PC062
 Acioli, T.PC048
 Adekunbi, D.A.PC065*
 Adeneye, A.A.PC023*
 Adeniyi, O.S.PC008*
 Adeoye, A.O.PC066
 Adeyemi, O.O.PC023
 Agbaje, E.O.PC023
 Agnew, T.PC063*
 Ahmed, R.U.PC060
 Ahmed, Z.PC004
 Akalestou, E.PC018*
 Akinboboye, O.O.PC065
 Akindele, A.J.PC004
 Akintayo, C.O.PC011*, PC041*
 Akomolafe, R.O.PC008
 Albuquerque, F.L.PC048
 Alcin, E.PC002
 Almond, K.PC049
 Alsaif, S.PC022
 Aluko, E.O.PC065
 AMABEBE, E.PC001*
 Amer, M.PC062
 Andrew, D.PC012
 Arms-Williams, B.PC029
 Ashcroft, F.M.PC012
 Ashour, F.PC062
 Astbury, S.PC024*
 Attwa, M.PC062
 Ayodele, M.J.PC041
 Aziz, S.A.PC061*

B

Bailey, D.M.PC047, PC051*
 Baker, L.PC007
 Bartsch, P.PC051
 Bell, R.PC024
 Bennett, K.A.PC044*
 Bentley, D.PC014
 Bhatta, A.PC067*
 Bikker, P.PC049
 Birtwistle, M.PC026, PC027*, PC053
 Blake, A.PC068
 Bloom, S.PL001*
 Bloom, S.R.PC018
 Blundell, J.E.PL003*
 Bolarinwa, A.F.PC021
 Bonnema, A.PC010
 Bosscher, D.PC010
 Botonis, P.PC031

Bourwis, N.PC015, PC058
 Bowl, M.PC063
 Bradley, C.PC007
 Bramham, K.PC038
 Brennecke, S.PC064
 Briley, A.PC038
 Brito-Gomes, J.PC048
 Brown, A.PC005
 Brown, R.C.PC029, PC030
 Brugniaux, J.V.PC047
 Buckley, M.M.PC068*
 Budge, H.PC026, PC027, PC043, PC053
 Bulmus, O.PC002
 Butler, T.PC064

C

Caldwell, R.PC067, PC067
 Canpolat, S.PC002
 Caridis, A.PC003
 Chan, E.PC064
 Chan, L.F.PC050
 Chan, S.PC014
 Charalambous, M.PC009, PC059*
 Christakis, I.PC018
 Christian, H.C.PC030
 Clark, A.PC015*, PC050
 Cleaton, M.A.PC009*
 Clifford, B.PC006
 Clifford, L.PC056
 Cohen, H.PC020
 Coleman, H.PC064
 Coll, T.P.PC050
 Coppock, R.PC044
 Costa, M.C.PC048*
 COSTA, M.C.PC045*
 Coursen, N.PC024
 Cox, R.PC063
 Cox, R.D.PC012
 Crooks, P.A.PC023
 Crouch, N.J.PC007
 Crowe, J.PC043
 Cryan, J.F.PC069
 Cryana, J.F.PC070

D

da Rocha, S.T.PC059
 Daley, A.SA015*
 Dalton, N.PC038
 Datta, A.PC040*
 Davey Smith, G.PC056

Davies, G. PC026, PC053
 Davies, J.S. PC030
 de Magalhaes, J.P. PC022
 Dempsey, Y. PC015
 Desforges, M. PC042
 Devlin, M. PC068
 Dinan, T.G. PC069
 Dodd, G.T. PC033, PC035
 Dolan, S. PC003, PC015, PC058*
 Douglas, J.A. PC007*
 Dowsett, L. PC037
 Duncan, S.H. PC010
 Dunichand-Hoedl, A. PC024

E

Elbaz, A. PC062*
 Elmes, M.J. PC006
 Enokela, P.O. PC008
 Erskine, R.M. PC055
 Eru, E.U. PC008
 Evans, W. PC047
 Everard, A. SA017*

F

Fainberg, H.P. PC049*
 Farquharson, F. PC010
 Ferguson-Smith, A.C. PC009, PC059
 Fernandes, M.S. PC045
 Fernandes, R. PC036*
 Flint, H.J. PC010
 Flynn, A. PC019*
 Ford, D. PC061
 Ford, S. SA007
 Fuenzalida, B. PC017
 Fyfe, C. PC010

G

Giannopoulou, I. PC031*
 Goff, L.M. PC019
 Goldsworthy, M. PC063
 Gomes, J.L. PC045
 Graham, A. PC003*, PC058
 Gratz, S. PC010
 Greenhaff, P.L. PC025
 Greenwood, S.L. PC042
 Guschina, I.A. PC030
 Gutman, R. PC020*

H

Halford, J. PC010
 Hall, A.J. PC044
 Hamilton-Sheild, J. PC056
 Hardie, G. SA012*
 Harrold, J. PC010
 Hesketh, J.E. PC061
 Hey, F. PC042*
 Hill, D. PC007
 Hodson, D.A. PC047*
 Holmes, A. PC022
 Homsy, M. PC038
 Hopkins, A.L. PC029, PC030
 Horton, N. PC022
 Hughes, J. PC044
 Hume, C. PC028*

I

Iranloye, B.O. PC016*

J

Jachs, B. PC028
 Jansson, J. SA010*
 Johnson, K. PC022
 Johnston, J.D. SA011*
 Johnstone, A. PC010

K

Kader, S. PC019
 Kandil, D. PC070*
 Karga, S.V. PC041
 Kelestimur, H. PC002*
 Kelly, J. PC010*
 Khan, M.A. PC060*
 Khanal, P. PC027
 Khodir, S. PC062
 King, J.A. PC007
 Kongsted, A.H. PC027
 Kostara, C. PC031

L

Laber, S. PC012*
 Lake, A.A. SA022*
 Lam, T. SA013*
 Larder, R. PC050
 Law, J. PC043*
 le Roux, C. PC051
 Leiper, J. PC037

Leiva, A.PC017
 Lelliott, C.PC050
 Leng, G.PC028, PC052
 Lewis, C.L.PC030
 Li, D.PC049
 Li, M.PC012
 Lim, E.SA003*
 Lloyd, J.SA018
 Logan, D.PC050
 Logue, J.SA004*
 Louis, P.PC010
 Luckman, S.M.PC033, PC035
 Ludwig, M.PC052

M

Maiti, K.PC064
 Mallinson, J.E.PC025
 Maric, A.PC032*, PC034
 Marimuthu, K.PC025
 Marley, C.J.PC047
 Mccoull, W.PC005
 McFarlane, E.PC007
 McKinnon, H.PC010
 McMullen, A.K.PC069
 McMullen, M.K.PC069
 McMullen, S.PC006
 McMurray, F.PC012
 Meisel, F.PC051
 Melo, R.P.PC045
 Menold, E.PC051
 Menzies, J.PC028
 Mercer, J.SA020*
 Merkestein, M.PC012
 Miras, A.SA008*
 Miwa, S.PC061
 Moir, H.J.PC013
 Morakinyo, A.O.PC004*
 Morgan, D.G.PC005*
 Morris, D.PC043
 Morse, C.I.PC055
 Mostyn, A.PC049
 Muir, R.PC006*
 Murton, A.PC025*
 Myers, M.G.PC033, PC035

N

Nathanielsz, P.W.SA007*
 Nielsen, B.PC024
 Nielsen, M.O.PC027
 Novoselova, T.PC050*

Nunn, N.PC033, PC035*

O

O'Rahilly, S.PC050
 O'Malley, D.PC068
 Ogunsola, A.O.PC065
 Ola-Davies, O.E.PC066*
 Olawuwo, O.S.PC066
 Oliveira, S.PC048
 Oliveira, S.M.PC045
 Osayande, S.I.PC001
 Otobo, T.M.PC054*
 Oyelowo, O.T.PC021*, PC065
 Ozanne, S.SA016*
 Ozcan, M.PC002
 Ozoene, J.O.PC001

P

Panagiotakos, D.PC031
 PAPADAKI, E.PC052*
 Pardo, F.N.PC017*
 Parkington, H.C.PC064*
 Pasupathy, D.PC038
 Patel, N.PC038*
 Patterson, S.PC057
 Paul, J.PC064
 Pears, C.PC063
 Pearson, G.PC055
 Peric Kacarevic, Z.PC032, PC034*
 Perry, V.PC026, PC053
 Pesci, I.PC010
 Petit, R.PC047
 Piper, S.E.PC037*
 Plagge, A.PC022*
 Pombo, J.PC036
 Pope, M.PC053*
 Popoola, A.A.PC065
 Poston, L.SA014*, PC019, PC036, PC038
 Poulton, J.PC063
 Price, M.PC042
 Pulbutr, P.PC014

Q

Quenby, S.PC006

R

Radford, E.PC059
 Radic, R.PC034

Radić, R.PC032
 Rafay, M.PC057*
 Raheela, K.PC006
 Raji, Y.O.PC011
 Rennie, M.J.PC025
 Rimmington, D.PC050
 Robinson, L.PC043
 Romero-Gonzalez, R.PC010
 Rooney, N.L.PC013*
 Roukens, R.PC051
 Ryan, S.PC010

S

Saez, T.PC017
 Sahin, Z.PC002
 Salsoso, R.PC017
 Samuel, R.PC044
 Samuel, S.E.PC066
 Samuelsson, A.SA006*, PC036
 Sanhueza, C.PC017
 Santos, J.C.PC045
 Sattar, N.SA001*
 Schellekens, H.PC069, PC070
 Scholtz, S.SA009*
 Selby, A.L.PC025
 Sellayah, D.PC012
 Seric, V.PC032
 Sheehan, P.PC064
 Shihab, H.PC056*
 Shirazi-Beechey, S.PC010
 Shmygol, A.PC006
 Silva, L.PC017
 Simpkin, A.PC056
 Skouroliakou, M.PC031
 Smith, K.PC025
 Smith, P.A.PC014*
 Smith, R.PC064
 Sobrevia, L.PC017
 Soffientini, U.PC003
 Song, A.PC024
 Soubry, A.SA005*
 Stakler, R.PC020
 Stathopoulou, M.PC031
 Statnick, M.A.PC033, PC035
 Steiner, T.PC051
 Stensel, D.J.PC007
 Stevenson, J.PC064
 Sun, Y.PC029
 Symonds, M.PC053
 Symonds, M.E.PC026, PC027, PC043,
 PC049

Szmigin, I.SA021*

T

Tamir, S.PC020
 Tarimobo-Otobo, R.PC054
 Taylor, P.D.PC036
 Taylor, R.PL005*
 Tedstone, A.SA019*
 Theeuwesb, W.F.PC069
 Tilston, T.W.PC029
 Timpson, N.SA002*, PC056
 Tomlinson, D.J.PC055*
 Tonta, M.A.PC064
 Torres-Fuentes, C.PC069*

U

Usher, S.PC012
 Uweru, O.J.PC016

W

Wade, K.PC056
 Wakeling, L.A.PC061
 Walker, M.PL002*
 Wells, L.H.PC029
 Wells, T.PC029*, PC030*
 Wessely, F.PC049
 Whiteley, S.L.PC013
 Wiens, F.PC026
 Winwood, K.PC055
 Woods, R.M.PC026*
 Worth, A.A.PC033*, PC035
 Wyatt, K.SA018*
 Wynn, E.PC050

Y

Yao, L.PC067
 Yeo, G.PL004*

Z

Zambrano, E.SA007
 Ze, X.PC010