AuPS/ASB Meeting - Adelaide 2010

Free communications: Endocrine signalling

Wednesday 1st December 2010 - Broughton Room - 09:30

Chair: Phil Poronnik

Periconceptional and early preimplantational undernutrition alters gene expression of metabolic and gluconeogenic regulating factors in the liver

S. Lie, J.L. Morrison, O. Wyss, S. Zhang and I.C. McMillen, Early Origins of Adult Health Research Group, Sansom Institute for Health Research, School of Pharmacy and Medical Sciences, University of South Australia, Australia.

Introduction: Maternal undernutrition during gestation can result in insulin resistance and glucose intolerance, leading to the development of type-2 diabetes. The effect of maternal undernutrition during the periconceptional period, however, has not been widely investigated. The metabolic master switch, AMP-Activated Protein Kinase (AMPK) and the master integrator of external signals, peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) play critical roles in liver metabolism. More importantly, the capacity of the liver for gluconeogenesis which is regulated by phosphoenolpyruvate carboxykinase, PEPCK, is critical in maintaining glucose homeostasis.

Hypothesis: We hypothesise that periconceptional (PCUN) and early preimplantational (PIUN) undernutrition will result in a decrease in gene expression of the metabolic regulators, AMPK and PCG-1α, as well as an increase in the gluconeogenic regulator, PEPCK in the fetal liver in late gestation.

Methods: Control ewes were fed 100% metabolisable energy (ME) from −45d to 6d after conception. Ewes in the PCUN group were fed 70% ME from −45d to 6d and ewes in the PIUN group were fed 70% ME from conception to 6d postconception. Liver samples were collected at 136-138d gestation. The mRNA expression of AMPK-α1, AMPK-α2, PCG-1α and the mitochondrial and cytosolic forms of PEPCK (PEPCK-M, PEPCK-C) were analysed using Real Time-PCR. $p<0.05$ was considered statistically significant.

Results: Hepatic mRNA expression of AMPK-α1 was decreased in the PIUN singleton compared to the control and PCUN groups. There was no difference, however, in the expression of AMPK-α2. The expression of PGC-1α and the cytosolic form of PEPCK was decreased in the PIUN and PCUN groups compared to the control group, with no difference in the expression of the mitochondrial form of PEPCK.

Conclusion: Periconceptional and early preimplantational undernutrition may result in a dysregulation of hepatic energy metabolism and a decrease in gluconeogenesis. The decrease in AMPK-α1 mRNA expression in the PIUN group may be due to a mismatch between the oocyte and the early embryo's energy status.

The effects of chronic moderate prenatal ethanol exposure on cardiovascular and renal artery function in adult rats

M. Tjongue,[1] M. Tare,[1] M.E. Probyn,[2] K.M. Moritz[2] and K.M. Denton,[1] [1]Department of Physiology, Monash University, VIC 3800, Australia and [2]School of Biomedical Sciences, University of Queensland, QLD 4072, Australia.

Maternal alcohol consumption during pregnancy remains common in society today. Prenatal exposure to high levels of alcohol can cause developmental abnormalities. The effect of more moderate alcohol exposure on the offspring remains unclear. An adverse environment in early life can increase the risk of cardiovascular disease in adulthood. The aim of this study was to examine the effects of chronic moderate fetal alcohol exposure on arterial pressure and vascular function in adult rat offspring. Female rats were given a complete liquid diet containing either ethanol (6% v/v equating to 15% of total calories and a peak blood alcohol content of 0.03 – 0.05%) or an isocaloric equivalent during pregnancy. Male (n = 6–9) and female (n = 7–8) offspring were studied at 1 year of age. Mean arterial pressure (MAP) was recorded under basal conditions and during restraint stress *via* radiotelemetry. At *post mortem*, the kidneys were removed and the renal interlobar arteries were isolated. Segments of renal interlobar artery were mounted onto a wire myograph for testing of smooth muscle and endothelial function. Arteries were bathed in warmed, oxygenated physiological saline (PSS). Other segments of artery were mounted onto a pressure myograph and bathed in 0mM Ca^{2+} PSS containing 1mM EGTA, for the assessment of passive wall stiffness. Basal MAP and heart rate (HR) were not different between vehicle and alcohol-treated groups. MAP and HR increased significantly in response to restraint stress, however, the increase in MAP was lower in alcohol-treated groups of both sexes (p = 0.001). Constriction of the renal interlobar artery evoked by single pulses of perivascular nerve stimulation was smaller in alcohol-treated females (p = 0.012), but not in the males. Vasoconstriction evoked by angiotensin II and phenylephrine, and vasodilation evoked by the nitric oxide donor sodium nitroprusside, were not altered with alcohol treatment. Total endothelium-dependent relaxation and the relaxation due to endothelium-derived hyperpolarizing factor were not different between treatment groups. Interlobar arteries from alcohol-treated females were modestly more compliant (p = 0.02), but arterial stiffness was not different between treatment groups for the males. In conclusion, this study demonstrates that even moderate maternal alcohol consumption, equivalent to 2 standard drinks per day, during pregnancy does have lasting effects on the cardiovascular system of the offspring.

Gonadotropin-inhibiting hormone (GnIH) regulates spontaneous action potentials in anorexigenic proopiomelanocortin neurons and orexigenic neuropeptide Y cells

J.S. Jacobi, H.A. Coleman, A. Sali, H.C. Parkington, M.A. Cowley and I.J. Clarke, Department of Physiology, Monash University, Clayton, VIC 3800, Australia..

Energy homeostasis and reproduction are intimately related and the mechanisms for such a close connection are the subject of considerable attention. However, our understanding of the neurobiological basis for this phenomenon is still incomplete. Neuropeptide Y (NPY) is a potent orexigen that is produced by cells in the arcuate nucleus of the hypothalamus. In the same nucleus, cells express the proopiomelanocortin (POMC) gene that produces melanocortins which are anorectic. Gonadotropin-inhibiting hormone (GnIH) peptide is a recently discovered inhibitory regulator of reproduction that is released by neurons localized in the dorsomedial nucleus of the hypothalamus, which is a nucleus with a role in regulating appetite and energy balance. It has been reported that central injections of GnIH increase food intake in birds and rats. Since GnIH neurons provide input to subsets of NPY and POMC cells, the aim of this study was to determine the effects of GnIH on the electrical activity of these appetite regulating neurons of the arcuate nucleus.

We used mice in which NPY or POMC genes were tagged with a transgene for renilla and green fluorescent protein. Mice were killed, the brain was rapidly removed into an ice slurry, and 250 μm thick coronal slices were cut. Slices were mounted in a recording chamber on the platform of an upright microscope and continuously superfused with artificial cerebrospinal fluid (aCSF) at 32°C. GFP-expressing cells were identified using epifluorescence, and patch electrodes were positioned using infrared DIC optics. Patch-clamp recordings of spontaneous action potential activity were made in whole-cell current-clamp mode or in cell-attached mode.

GnIH inhibited the firing rate of POMC cells. Since these cells are anorexigenic this inhibition may be involved in the increase of food intake induced by GnIH. The GnIH regulation of NPY cells was more complex. In one group of NPY cells, GnIH inhibited spontaneous action potential activity and this effect was associated with a clear hyperpolarisation of the membrane potential. Another group of NPY cells showed no effect of GnIH. The combined presence of blockers of glutamatergic and GABAergic receptors decreased spontaneous action potential activity. Under these conditions, GnIH evoked an increase in action potential activity.

In conclusion, these data indicate that GnIH, in addition to having an important role in regulating reproductive function, is also a significant regulator of the appetite/energy expenditure system within the hypothalamus.

Maternal overnutrition during the periconceptional period and gender influences insulin signalling and glucose handling in lambs after birth

L.M. Nicholas,[1] L. Rattanatray,[1] S. McLaughlin,[1] S.E. Ozanne,[2] B.S. Muhlhausler,[1] D. Kleeman,[3] S. Walker,[3] J.L. Morrison[1] and I.C. McMillen,[1] [1]Sansom Institute of Health Research, University of South Australia, SA 5000, Australia, [2]Institute of Metabolic Science-Metabolic Research Laboratories, Addenbrooke's Hospital, Cambridge, CB2 0QQ, United Kingdom and [3]Turretfield Research Centre, South Australian Research and Development Institute, SA 5000, Australia.

The increased prevalence of overweight and obesity amongst Australians aged 18 years and older is reflected in an increase in the number of women who are entering pregnancy obese (Callaway, O'Callaghan & McIntyre, 2009; Ryan, 2007; LaCoursiere *et. al.*, 2005). Maternal obesity before pregnancy is associated with an increased risk of obesity and metabolic disease in the offspring (Catalano *et. al.*, 2003). How the liver responds to insulin is an important contributor to the body's ability to maintain normal glucose levels throughout life (Postic, Dentin & Girard, 2004). Little is known, however, about the impact of maternal obesity or the impact of dieting before conception on how the liver of her offspring responds to insulin in order to maintain glucose homeostasis. The present study investigated whether maternal obesity during the periconceptional period (*i.e.* before and just one week after conception) resulted in changes in the expression of insulin signalling molecules and genes that control glucose output in the liver of postnatal lambs. This study also investigated the effects of dietary restriction in overnourished and normally nourished ewes on these measures of hepatic insulin sensitivity in the offspring.

Donor ewes were randomly allocated to one of 4 treatment groups. The CC group received a control diet of 100% metabolisable energy requirements (MER) for 4 months before conception. The CR group received a diet of 100% MER for 3 months followed by a restricted diet (70% MER) for 1 month. Ewes in the HH group, which is our model of maternal periconceptional overnutrition, was overnourished (~180% MER) for 4 months. The HR group, which is our model of dietary restriction in the overnourished ewe, was overfed for 3 months followed by a restricted diet of 70% MER for 1 month. After conception, single embryos were transferred into non obese 'recipient' ewes at 6-7 days after conception. Ewes lambed normally and tissues including the liver were collected at 4 months of age for analysis.

There was a lower ($p<0.05$) abundance of insulin signalling proteins Akt2, pAkt and pFoxO1 in the HH group and this effect was ameliorated in the HR group. Interestingly, expression of the gluconeogenic enzyme PEPCK (mitochondrial form) and 11βHSD1 mRNA was lower ($p<0.05$) in the HH group and this effect was also abolished in the HR group. IN addition, expression of the cytosolic form of PEPCK mRNA was lower ($p<0.05$) in CR, HH (male lambs) and HR groups.

In conclusion, periconceptional overnutrition appears to program decreased expression of insulin signalling molecules in the liver of the offspring which could contribute to the emergence of insulin resistance in later life. In contrast, periconceptional over- and under-nutrition differentially suppress the mitochondrial and cytosolic forms of the major gluconeogenic enzyme in the liver which may protect the lamb from the consequences of poor insulin signalling in the immediate term.

Callaway, L.K., O'Callaghan, M.J., & McIntyre, H.D. (2009). Barriers to addressing overweight and obesity before conception. *Medical Journal of Australia* **191:** 425-428.

Catalano, P.M., Kirwan, J.P., Haugel-de Mouzon, S., & King, J. (2003). Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. *Journal of Nutrtrition* **133:** 1674S-1683S.

LaCoursiere, D.Y., Bloebaum, L., Duncan, J.D., & Varner, M.W. (2005). Population-based trends and correlates of maternal overweight and obesity, Utah 1991-2001. *American Journal of Obstetrics and Gynecology* **192:** 832-839.

Postic, C., Dentin, R., & Girard, J. (2004). Role of the liver in the control of carbohydrate and lipid homeostasis. *Diabetes and Metabolism* **30:** 398-408.

Ryan, D. (2007). Obesity in women: A life cycle of medical risk. *International Journal of Obesity* **31:** S3-S7.

The role of regulator of calcineurin 1 (RCAN1) in the regulation of glucose homeostasis

H. Peiris,[1] D. Mohanasundaram,[2] J. Brealey,[3] C. Jessup,[2,5] T. Coates,[2,5] M. Pritchard[4] and D. Keating,[1]
[1]Molecular and Cellular Neuroscience Group, Department of Human Physiology and Centre for Neuroscience, Flinders University, Adelaide, SA 5000, Australia, [2]Central Northern Adelaide Renal and Transplantation Service, Royal Adelaide Hospital, North Terrace, Adelaide, SA 5000, Australia, [3]Surgical Pathology, Electron Microscopy Unit, Royal Adelaide Hospital, North Terrace, Adelaide, SA 5000, Australia, [4]Department of Biochemistry and Molecular Biology, Monash University, VIC 3800, Australia and [5]School of Medicine, University of Adelaide, Adelaide, SA 5000, Australia.

Regulator of calcineurin 1 (RCAN1) is a gene located on chromosome 21 and is over expressed in the brains of Down syndrome (DS) patients. Our lab has previously shown that RCAN1 regulates exocytosis in adrenal chromaffin cells. As the incidence of diabetes is 5-10 times greater in the DS population we are investigating the effect of increased RCAN1 expression and its possible role in the pathogenesis of diabetes. Transgenic mice with a universal over-expression of RCAN1 were generated for this study. *In vivo* studies indicate that transgenic mice develop age-dependent diabetes characterized by increased fasting blood glucose levels of 5.8±0.3 mmol/L (n=9) at 60 days old compared to 4.2±0.2 mmol/L (n=9) in age-matched wild-type mice ($p<0.05$). Glucose tolerance, measured by injecting 2mg glucose/g body weight, is also reduced in transgenic mice, with glucose values reaching peak levels of 27.5±1.4 mmol/L (n=5) after 60 minutes compared to 19±1.3 mmol/L (n=5) in wild-type mice ($p<0.01$). Immunohistochemical analysis of pancreatic islets revealed that transgenic mice have a 70% reduction in islet area (n=4) at 100 days. Electron microscopy analysis reveals that transgenic mice have a 40% increase in empty secretary vesicles (n=3) at 120 days. Transgenic mice also have significantly decreased fasting blood insulin values at 120 days (n=6) when compared to age-matched wild-type mice. In islets of transgenic mice, expression of genes such as those mutated in hereditary forms of monogenic type 2 diabetes (MODY) and others related to β-cell survival and insulin production were downregulated. Our findings highlight a novel role of RCAN1 in regulating glucose homeostasis, islet growth, secretary vesicle loading and insulin release. Additionally expression of RCAN1 increased 2.5 fold ($p<0.05$) when islets were exposed *in vitro* to 16.7 mM glucose for 6 days. This along with our previous findings provide the exciting proposition that RCAN1 may be involved in the β-cell failure and hypoinsulinemia that occurs in the later stages of type 2 diabetes.