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The effects of birth weight and postnatal growth rate on the development of type 2 diabetes in pigs

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Epidemiological studies have linked small birth weight and rapid compensatory growth to a number of chronic diseases, such as type 2 diabetes, hypertension and cardiovascular disease. We used Yucatan miniature pigs to determine if overfeeding during compensatory growth contributes to the development of chronic diseases, including type 2 diabetes. Runt piglets (<800g) were paired with the largest same sex littermate (>1100g) and taken from the sow at 3 days of age (N=12). During the first 4 weeks, piglets were fed milk replacer ad libitum; thereafter pigs were fed standard chow ad libitum. In order to monitor type 2 diabetes development, blood samples were taken serially and analyzed for glucose, insulin, triglycerides, cholesterol, LDL, and HDL. At 8 months of age, pigs were surgically fitted with venous catheters. After recovery, intravenous glucose tolerance tests (IVGTT), and insulin sensitivity tests (IST) were performed. At 9 months of age pigs were killed and the pancreas was removed for estimation of beta cell mass. During formula feeding runts (N=12) demonstrated compensatory growth, but were still smaller than littermates ($p<0.05$) by 4 weeks of age. By 8 months of age (N=8), runts (62.58+/-6.59) had completely caught up in body weight to their littermates (67.51+/-4.69). Preliminary results (N=6) of fasting glucose during the IVGTT and IST were not different between runts and littermates. Using the miniature pig, we have successfully established a model for compensatory growth that can be used to investigate the mechanisms of early origins of adult disease. (Supported by CIHR and NSERC).

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Argpyrimidine: A Novel Biological Antioxidant

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Argpyrimidine, a major end-product of non-enzymatic protein glycation by methylglyoxal has been implicated in the pathophysiology of diabetes and neurodegenerative diseases. Chemically argpyrimidine is a substituted pyrimidinol, with structural features common to known antioxidants. The objective of this study was to investigate the ability of argpyrimidine to function as an antioxidant. Chemical synthesis of argpyrmidine was accomplished by reacting L-arginine with 3-acetoxypentane-2,4-dione under acidic conditions and purification of the product by column chromatography. Argpyrimidine inhibited lipid-peroxidation of rat-brain homogenates catalyzed by hydroxyl radicals, metal-ions and autoxidation in a concentration- and time- dependent manner suggesting that argpyrimidine functions as a chain-breaking antioxidant. Argpyrimidine scavenged oxygen free radicals, intracellular hydrogen peroxide and inhibited the free-radical-mediated nicking of plasmid-DNA. Taken together, these data suggest that argpyrimidine may function as an antioxidant and may therefore have biological relevance in pathophysiologies associated with diabetes and neurodegenerative diseases.

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Obesity Augments the Formation of Angiotensin II-induced Abdominal Aortic Aneurysms

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Numerous studies have demonstrated the severe risks obesity poses for cardiovascular health, including hypertension and coronary artery disease. Previous studies demonstrate that chronic infusion of Angiotensin II (AngII) to hyperlipidemic LDL receptor -/- mice fed a Western diet consistently (80% incidence) promotes the formation of abdominal aortic aneurysms (AAA). In contrast, infusion of AngII to normolipidemic C57BL/6 mice results in a low incidence (10%) of AAA formation. We sought to determine if diet-induced obesity will increase the incidence and/or severity of AngII-induced AAAs. Male C57BL/6 mice were fed a normal laboratory diet (Normal; 5% kcal as fat) or a high fat diet (HF; 60% kcal as fat) for 15 weeks. Subsequently, osmotic mini pumps were implanted subcutaneously to deliver AngII at a constant rate (1,000ng/kg/min) for 28 days. Body weight and blood pressure were monitored throughout the study. HF-fed mice exhibited an increase in body weight compared to Normal-fed mice (Normal: 33.9 ± 0.8 vs. HF: 38.9 ± 1.4 g; $P<0.05$) with an increase in adipose mass (adiposity index of Normal: 4.4 ± 0.3 vs. HF: 6.8 ± 0.4 %; $P<0.05$). AngII-induced elevations in blood pressure were similar between groups. Total cholesterol concentrations in sera were not significantly different in mice that did or did not exhibit AAAs. The incidence of AAA formation was increased in HF-fed mice compared to Normal (Normal: 22.2 vs. HF 76.5%; $P>0.05$), with a significant increase in adiposity index in mice that exhibited AAAs. Interestingly, periaortic vascular adiposity differed between HF- and Normal-fed mice, as did the composition of perivascular adipose tissue in the thoracic versus abdominal aorta. This study suggests a role for obesity to promote AAA formation. *USDA 2005 38420 15825*

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Compensatory Growth And Blood Pressure Using Non-Invasive And Telemetry Techniques In Yucatan Mini-Pigs

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In epidemiological studies, low birth-weight followed by catch-up growth is associated with an increased risk of hypertension and cardiovascular disease in adulthood. Runt Yucatan miniature pigs (<800g) were paired with the largest same sex littermate (>1000g) and fed milk replacer ad libitum from 3 to 31 days of age; thereafter they were fed standard chow ad libitum. During the first 4 wk, runts demonstrated catch-up growth and by 8 months of age their body weights were not different than their littermates. In order to monitor hypertension, we used the noninvasive blood pressure (NIBP) technique using a foot cuff at 8 months of age. We subsequently measured BP using telemetry. With NIBP, systolic, diastolic and mean arterial pressure (MAP) (runt: 93 ± 12, littermate: 92± 10 mmHg) were not different between groups. However, for all pigs, none of these data correlated with respective telemetry data. NIBP heart rate tended to correlate with peak heart rate using telemetry ($P=0.08$) but blood pressure estimates tended to be negatively correlated ($P=0.11$). Using telemetry data for all pigs, unimodal distributions over 24 h were observed for MAP (117± 7 mmHg), heart rate (87± 5 bpm), systolic (140± 7 mmHg), diastolic (97± 7 mmHg) and pulse (43 ± 6 mmHg) pressures. Although blood pressure parameters were not different between runts and littermates, relative size of left ventricles in runts were larger than littermates. BP telemetry data during a salt challenge will also be presented. We have established a pig model of compensatory growth which can be used to further investigate early origins of cardiovascular disease (Supported by CIHR).

CARDIOVASCULAR PHARMACOLOGY

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Expression of a recombinant A1 domain of human VWF in COS 7 cells for conformational studies

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Von Willebrand factor (VWF) is a multimeric, plasma glycoprotein that plays an important role in hemostasis and thrombosis. It promotes platelet adhesion to damaged vascular endothelium. The A1 domain in VWF contains multiple binding sites, including those for platelet glycoprotein Ib, heparin, and the artificial modulators ristocetin and botrocetin. The structure of this domain is critical to its function as several point mutations have been found within this domain in patients with type 2 von Willebrand disease (VWD). Conformational changes in the domain are a topic of intense interest. Differences in its structure as a result of natural mutations in VWD have been demonstrated by X-ray

crystallography of recombinant A1 domain fragments. Such studies, however, do not prove that the native A1 domain can undergo a change in conformation. We plan to examine the patterns of digestion of a recombinant A1 domain by a various proteases in the presence and absence of ristocetin to test this hypothesis. In the present study, we have cloned the A1 domain of human VWF from genomic DNA and have designed a construct with a cleavable hexahistidine tag to express the recombinant protein. This facilitates purification of the glycosylated protein after its expression in COS 7 cells. Cleavage of the tag will allow subsequent studies of conformational change to be performed with the protein in its native state.

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Differences in the native and heparinase I digestion profiles of a generic enoxaparin: Pharmacologic implications

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Several generic versions of the low molecular weight heparin enoxaparin (Lovenox, Sanofi-Aventis, Paris, France), have recently become available globally. Some of these are undergoing regulatory review in the U.S. Although the mean molecular weight and anti-Xa potency of these agents may be comparable to the branded product, the distribution of oligosaccharide components may differ. To investigate the distribution of oligosaccharide components of Lovenox and a generic version, Dripanina (Ariston, Sao Paulo, Brazil), gel-permeation chromatographic profiles of the native products and their heparinase I digested oligosaccharides were compared. Both products were digested using 0.1 U/ml heparinase I in a calcium-supplemented buffer system. The native oligosaccharide profiles showed comparable molecular weight patterns. However, Dripanina exhibited a much greater prevalence of a disaccharide component (>40%) and significant salt contamination (>10%) compared to Lovenox. Upon heparinase I digestion, the distribution profile of oligosaccharide components in Lovenox and Dripanina differed markedly. The oligosaccharide components with a molecular weight >2.5 kDa were similar in both drugs. However, Dripanina contained a higher proportion of oligosaccharides with molecular weight <2 kDa (19%) and <1 kDa (24%). These results demonstrate the significant differences in the oligosaccharide components between Lovenox and a generic version, Dripanina, despite claimed equivalence. This observation underscores the importance of clear guidelines on molecular profile specifications of the component oligosaccharides.

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Defibrotide does not cross-react with HIT antibodies. Implications in the management of HIT

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Defibrotide is a mammalian DNA derived antithrombotic and anti-ischemic agent that does not produce systemic anticoagulation. Heparin-induced thrombocytopenia (HIT) is an immune disease related to heparin exposure, in which patients are at risk of developing life- and limb-threatening thrombosis. Studies were performed to determine whether defibrotide cross-reacts with HIT antibodies formed. Sera from 141 clinically confirmed HIT patients were tested for platelet activation in the presence of defibrotide (1-100 μg/ml) and unfractionated heparin (1-100 μg/ml). 103 sera (73%) produced platelet aggregation and serotonin release activities with heparin. Only 2 samples (1%) showed a weak reactivity with defibrotide which was eliminated by heparinase treatment indicating heparin contamination in the patient sample. Further studies revealed that defibrotide does not complex with PF4, and that prolonged incubation of defibrotide with platelet rich plasma does not mobilize PF4. Studies of patients treated with extended dosing of intravenous or oral defibrotide (n=270) demonstrated that HIT antibodies are not generated with defibrotide treatment. Taken together these studies suggest that defibrotide may be a useful antithrombotic agent for the management of patients with HIT. Defibrotide would not promote the HIT pathology of platelet activation; it would suppress the hypercoagulable state associated with HIT; and it is orally bioavailability making it applicable for both acute and long-term treatment.

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Molecular characterization of Kv2.1 channels in sheep vasculature: Role of Kv channels in regulating vascular tone

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The voltage-gated potassium channel 2.1 (Kv2.1) is regarded as one of the primary oxygen sensitive potassium channels in the pulmonary circulation. The dilator function of Kv2.1 is enhanced in isolated sheep conduit compared to resistance pulmonary arteries, and may be increased during development of the same animal. However, neither the functional role nor distributions of Kv2.1 in other vasculatures have been described in detail. Thus, the goal of this study was to use RT-PCR and tension-recording to compare the transcript expression and dilator influence of Kv2.1 channels between isolated sheep conduit and resistance pulmonary, coronary and renal arteries. Using human Kv2.1 channel primers, we demonstrated the expression of Kv2.1 channel mRNA in all vascular preparations. 4-Aminopyridine (4-AP) (0.001-3 mM), a nonspecific blocker of Kv channels, produced concentration-dependent contractions of conduit pulmonary and renal arteries. On the other hand, 4-AP had no significant effect on the basal tone of isolated sheep resistance artery and coronary arteries. These results suggest that Kv channels may play an important role in regulating the tone of sheep conduit and renal arteries, but they do not appear to contribute to the regulation of basal tone in resistance pulmonary arteries and coronary arteries. The latter observation raises the possibility that Kv2.1 channels are not the primary contributor to hypoxic pulmonary vasoconstriction in sheep. IVRI research support, India.

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Aldosterone Regulation of the Cardiac Sodium-Calcium Exchanger

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The objective of this study was to determine whether the protein expression and functional activity of the cardiac sodium-calcium exchanger was subject to regulation by activation of mineralocorticoid receptors. The studies were carried out in cultured adult rat ventricular myocytes. Protein expression was evaluated by Western blotting, using a monoclonal antibody to the NCX1 protein (R3F1, Swant, Switzerland). Functional activity of the sodium-calcium exchanger was evaluated by first inducing reverse-mode exchanger activity and then using fluorescent calcium indicators to measure changes in cytosolic calcium. Aldosterone strongly inhibited exchanger protein expression in a dose-dependent manner (0.1-10 nM). This effect depended on mineralocorticoid activation, as it could be blocked by spironolactone. Cytosolic calcium measurements confirmed that the decreased protein expression seen with 10 nM aldosterone was accompanied by a significant inhibition of sodium-calcium exchanger functional activity. This work was supported by NHLBI R01HL056910 (RWH).

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Adenosine A2A receptor plays a pivotal role in mediating clonidine hypotension in aortically barodenervated rats

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In a previous study, we have shown an important role of central adenosine in the hypotension caused by clonidine, a mixed α_2/I_1 centrally acting antihypertensive agent. Intracisternal (i.c.) 8-sulfophenyl theophylline (8-SPT) abolished, while dipyridamole enhanced, clonidine-evoked hypotension in conscious aortic barodenervated (ABD) rats. Since clonidine is a mixed α_2/I_1, we investigated whether the I_1- or the α_2-mediated hypotension is dependent on central adenosine signaling. To this end, conscious ABD rats were instrumented 5 days earlier for femoral blood pressure recording and i.c administration of selective I_1 (rilmenidine 25 μg/kg;n=6-7) or α_2 (α-methlnorepinephrine, αMNE, (4μg/kg; n= 6-7) agonist 30 min after central (i.c) adenosine receptor blockade (8-SPT; 10 μg/kg) or aCSF. The hypotensive response