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Wyndham Competition (Student Presentations) Sessions

Detection of serum antibodies to the human coronavirus-NL63 nucleocapsid protein

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The outbreak of the SARS coronavirus in 2003 showed that, what was once thought of as merely a common cold virus was capable of undergoing recombination to result in a much more lethal form of the virus. Shortly after the SARS-CoV outbreak, another human coronavirus (HCoV-NL63) was identified. This virus has since been shown to have a worldwide distribution and is continuously circulating in the human population. The primary function of the coronavirus nucleocapsid (N) protein is the formation of the ribonucleocapsid core by interacting with viral RNA during virus assembly. The nucleocapsid protein is also highly antigenic and highly expressed during viral infection making it suitable for the development of diagnostic assays. In this study, an antibody-capture ELISA assay was developed using recombinant clones of the full length HCoV-NL63 (N1: aa 1-378) N protein, as well as truncated clones corresponding to the N- (N2: aa 1-189) and C-terminals (N3: aa 190-378) of the NL63 N-protein. The ELISA was used to screen human serum samples for the presence of HCoV-NL63 N-specific antibodies. Also, the mutants were included to map the immunogenic epitope of the HCoV-NL63 N-protein. Even though serum antibodies to HCoV-NL63 N protein were detected, cross-reactivity between SARS-CoV N protein and the serum HCoV-NL63 antibodies were also observed. Interestingly, initial data mapped the antigenic site to both the N- and C-terminal portion of the nucleocapsid protein. Some cross-reactivity to another coronavirus N protein was observed and the assay needs to be optimized.

Key words: SARS, human coronavirus, HCoV-NL63, ribonucleocapsid core, map immunogenic epitope.

Human coronavirus-NL63 nucleocapsid protein-induced immune response in human whole blood cultures

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Human coronavirus-NL63 (HCoV-NL63) was first isolated in 2003 from the nasal aspirate of an infant. This virus is closely related to the severe acute respiratory coronavirus (SARS-CoV), but unlike SARS-CoV, HCoV-NL63 continuously circulates in the human population. Recent studies have focused on the effect of coronavirus infections on the host immune response, in particular the cytokines produced in response to these virus infections. Coronavirus nucleocapsid (N) protein is a multifunction protein and plays an important role in viral assembly and pathogenesis. In this study, the effects of HCoV-NL63 N protein on the cytokines that regulate immune responses in lymphocytes were determined. Full-length as well as deletion-mutants of HCoV-NL63 N protein were expressed in *Escherichia coli* KRK cells and column-purified. Cellular responses induced by these recombinant N proteins were evaluated in whole blood culture. A double antibody sandwich ELISA assay was used to measure the immune responses; interferon-gamma (IFN-γ), interleukin-10 (IL-10) and interleukin-6 (IL-6) responses against the N proteins was observed. While lymphocyte activation resulted in high expression levels of IL-6, IFN-γ and IL-10 cytokines were secreted at much lower levels. This study showed that the HCoV-NL63 N protein elicits a broad based cellular immune response.

Key words: Human coronavirus-NL63, cytokines, interferon-gamma (IFN-γ), interleukin-10 (IL-10) and interleukin-6 (IL-6).

Whole body vibration increases hip and preserves spine bone mineral density in well-trained road cyclists

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Road cycling is associated with low bone mineral density. Whole body vibration therapy could potentially increase the bone mineral density of cyclists. We determined the effects of ten weeks of whole body vibration training, ten minutes per day, three times per week on the bone health of a group of well-trained cyclists (n = 11) who also continued with their normal cycling training during the intervention period. Another well-trained group of cyclists (n = 7) served as a matched control group and did not receive whole body vibration training, but continued with their normal cycling training for ten weeks. In addition, the cyclists were matched with 19 sedentary controls. At baseline, all participants underwent regional dual x-ray absorptiometry scans to measure bone mineral density, and completed informed consent forms, a Calcium Rapid Assessment questionnaire and training history questionnaires. At baseline, both cycling groups had lower pelvis bone mineral density ($p<0.05$) and higher head bone mineral density ($p<0.05$) than the sedentary controls but were otherwise well matched. All cyclists were reassessed after ten weeks of training. At the reassessment, we found that cyclists in the vibration group got significantly taller ($p = 0.038$). They also increased their hip bone mineral density by 1.65% on average ($p = 0.026$). The control group showed a decrease in spine bone mineral density (-0.94%, $p = 0.020$) which was not seen in the vibration group. Lean body mass was correlated with bone mineral density at most sites ($p<0.05$). Whole body vibration increases hip and preserves spine bone mineral density in road cyclist after ten weeks of training and therefore shows promises as a means of attaining improvements in bone density in road cyclists who are at increased risk for the development of osteoporosis.

Key words: Cycling, vibration, therapy, bone density, training, improvement, hip bone.

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The efficacy of *Prosopis glandulosa* as antidiabetic treatment in rat models of diabetes and insulin resistance

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Diabetes mellitus is increasing and the need for therapeutics is crucial. In recognition of this, untested antidiabetic agents are flooding the market. Diavite™ (product consisting solely of dried/ground pods of *Prosopis glandulosa)* is currently marketed as a supplement with glucose stabilizing properties. However, these claims lack scientific evidence. The aim of this study was to determine the efficacy of *P. glandulosa* as an antidiabetic agent. Male Wistar rats were rendered (a) type 1 diabetic after an intraperitoneal injection of STZ (40 mg/kg) and (b) insulin resistant after 16-week high caloric diet (DIO). Half the animals were placed on *P. glandulosa* treatment (100 mg/kg/day) for 8 weeks and the remaining animals served as controls. At the time of sacrifice, blood was collected for glucose and insulin level determination, the pancreata of the STZ rats were harvested for histological analysis and cardiomyocytes and skeletal muscle strips prepared from control and DIO animals for determination of insulin sensitivity. Type 1 diabetes: *P. glandulosa* treatment resulted in significant increased insulin levels ($p < 0.001$), accompanied by a significant decrease in blood glucose levels ($p < 0.05$). Additionally, *P. glandulosa* treatment resulted in increased small β-cells ($p < 0.001$) in the pancreata. The body weight of the STZ animals decreased significantly after STZ injection, with *P. glandulosa* treatment partially preventing this. *DIO insulin resistance*: *P. glandulosa* treatment resulted in an increased basal ($p < 0.01$) and insulin-stimulated ($p < 0.05$) glucose uptake in cardiomyocytes. Additionally, *P. glandulosa* treatment also resulted in increased insulin-stimulated ($p < 0.05$) glucose uptake in skeletal muscle from the treated control group. The present study showed that *P. glandulosa* treatment moderately lowers glucose levels in different animal models of diabetes, stimulates insulin secretion, leads to the formation of small β-cells and improves insulin sensitivity of skeletal muscle strips and isolated cardiomyocytes.

Key words: Antidiabetic agents, glucose, insulin, formation, small β-cells, cardiomyocytes.

The effects of *Syzygium aromaticum*-derived oleanolic acid in kidney and liver cell lines, and on kidney function of male Sprague-Dawley rats

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Studies indicate that *Syzygium* spp derived oleanolic acid (3ß-hydroxy-olea-12-en-28-oic acid, OA) possesses hypoglycaemic and kidney ameliorative properties in streptozotocin-induced (STZ) diabetic *rats*. However, the shortfall of this study is that cytotoxic effects of this extract were not investigated; moreover, these OA properties were establish-

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ed with a single dose. In *vitro* cell culture studies are useful for prediction of toxicity of therapeutic drugs in specific organs. Therefore, this study investigated the cytotoxic effects of OA in human hepatocellular carcinoma (HepG2), human embryonic kidney (HEK293) and kidney distal convoluted tubule bovine (MDBK) cell lines. Thereafter, the study investigated the effects of this triterpene in renal fluid and electrolyte handling using various concentrations. OA was extracted using a previously validated protocol in our laboratory. Sub-chronic doses of OA were administered to male Sprague-Dawley rats twice (8h apart) every third day for five weeks. Rats treated with deionised water served as controls. Cytotoxic effects of various OA concentrations were investigated in human hepatocellular carcinoma (HepG2), human embryonic kidney (HEK293) and distal convoluted tubule (MDBK) cell lines using the 3-4,5 dimethylthiazol-2-yl-2,5diphenyltetrozolium bromide (MTT) assay. All data are presented as means ±SEM. By comparison with control animals, OA significantly ($p<0.05$) increased Na^+ excretion with a concomitant decrease in K^+ excretion rate from week 3 to 5 week without influencing the volume of urine voided. OA treatment decreased plasma creatinine concentrations with concomitant increase in glomerular filtration rate (GFR) at the end of the experimental period. Furthermore, OA treatment significantly decreased the mean arterial pressure from week 3 to 5. The cell viabilities of HepG2, HEK293 and MDBK cell lines were significantly increased after 24 h exposure, whereas the HEK293 and MDBK viabilities dropped after 72 h. This could be attributed to depletion of nutrients in the culture medium. Overall, previous observations of the anti-diabetic properties of OA and the current results which show that OA does not exhibit toxicity in kidney and liver cell lines suggest that the triterpene may be a beneficial anti-diabetic agent.

Key words: Oleanolic acid, triterpene, antidiabetic, no cytotoxic effects, kidney, liver, cell lines.

Effects of *Syzygium aromaticum* derived oleanolic acid on glucose transport across the rat-everted intestinal sacs *in vitro*

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Studies in our laboratory show that the *Syzygium aromaticum* derived triterpene, oleanolic acid (OA), exerts hypoglycaemic effects in part via increased glycogen synthesis in the liver and skeletal muscle. Since the small intestine expresses glucose-6 phosphatase (Glc6Pase), the last enzyme of gluconeogenesis and glycogenolysis, we hypothesized that OA influences glycogen synthesis within the intestinal walls. Accordingly, this study investigated the effects of OA on glucose transport using the rat-everted intestine and gut wall glycogen concentration to further elucidate the mechanism(s) of hypoglycaemic effects of the triterpene. Male Sprague-Dawley rats (250-300g body weight) were sacrificed following an 18h fast. The small intestine was removed, washed, cut into segments and everted. The empty sac was filled with 1 ml of Krebs-Henseleit bicarbonate (KHB) buffer. The distended sac was placed inside the organ bath containing 50 ml of the same incubation medium (mucosal solution). D-Glucose (10 mM) was added to the medium just before the start of the experiments. Graded concentrations of OA (0.37, 0.75, 1.5, 3.5 mg/ml) were added to the mucosal solution in each group). The effects of OA were compared with the standard drug phlorizin (2.5 mg/ml). Control intestinal sacs were administered an equal volume of the vehicle at the corresponding time. The active transport of D-glucose was evaluated by measuring the changes in concentration of the glucose inside and outside the intestinal sacs after the 30 min incubation period. Glycogen concentration was determined in small intestine mid portion segments isolated from rats with or without food after 18 h OA (80 mg kg^{-1}, p.o.) administration. Glycogen was determined in the intestinal tissues using the protocol previously used in our laboratory. All data was expressed as means ±

standard error of means (SEM). Statistical analysis was done using GraphPad Instant Software. Values of $p<0.05$ were considered statistically significant. OA produced a dose-dependent decrease of glucose transport from the mucosa to the serosa as well as an increase in glycogen concentration within the walls of the small intestine. Phlorizin also produced a dose dependent decrease of glucose transport across the small intestine. These results suggest that the hypoglycaemic properties of OA are mediated, in part, via the inhibition of glucose transport across the small intestine with an increase in glycogen concentration within the intestinal walls.

Key words: Oleanolic acid, glycogen, muscle, liver, glucose transport, walls intestine, rats.

The signaling pathways involved in the induction of cell death in breast cell lines by a novel antimitotic and anticarbonic anhydrase IX compound

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A new antimitotic, C19, was synthesized by Ithemba Pharmaceuticals. Ligand binding assays revealed that the compound reversibly binds to CAIX (453 nM) at lower concentration than CAII (655 nM), indicating that it is a potential antimetastatic agent. Growth studies indicate that the compound is 5 times more potent when compared to a similar phase II antimitotic, 2-methoxyestradiol. The effects of C19 on cell growth, apoptosis, reactive oxygen species formation, morphology, gene expression and protein expression in metastatic breast adenocarcinoma MDA-MB-231 cells, tumorigenic breast adenocarcinoma MCF-7 cells and non-tumorigenic MCF-12A breast cells were conducted. Microarray studies determined that the gene expression of a common set of genes was affected in all three cell lines indicating a shared mechanism of action. These include genes associated with cell cycle control, apoptosis, microtubule dynamics and response to reactive oxygen species. There are also different genes affected in each cell line indicating a divergence from the shared mechanism of action. Antibody arrays demonstrated that caspase 7 was up-regulated in MDA-MB-231 cells and not MCF-7 cells and the p96 diasabled homolog 1 was up-regulated in MCF-7 cells and not MDA-MB231 cells. C19 also induced an increase in apoptosis in the cell lines. This increase in apoptosis was partially inhibited by the selective p38 MAP kinase inhibitor, SB239063, in MDA-MB-231 cells and not MCF-7 and MCF-12A cells. The selective JNK kinase inhibitor, SP600125, in turn partially inhibited apoptosis induction in all three cell lines; however it was more pronounced in the MCF-12A and MCF-7 cells. While a common mechanism is suggested by the gene expression studies, the protein expression and apoptosis assays suggest a divergence and different pathways are activated in different cell lines. The importance of these results will aid the development of treatments of cancer targeted at specific pathways associated with specific cancers.

Key words: Antimitotic, anticarbonic anhydrase IX, apoptosis, gene expression, protein expression.

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Effects of *Syzygium aromaticum*-derived maslinic acid on blood glucose of streptozotocin induced-diabetic rats

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Syzygium spp. extracts contain methyl malsinates and oleanolic acid (OA) triterpene mixtures which have been shown to lower blood glucose of streptozotocin (STZ)-induced diabetic rats. Maslinic acid (MA) has, however, been shown to lower blood glucose in some experimental diabetic models excluding diabetes type 1. Accordingly, this study was designed to investigate the influence of MA on blood glucose concentrations of STZ-induced diabetic rats, a model for type 1 diabetes. MA was extracted from *Syzigium aromaticum* clove flower buds with dichloromethane and ethyl acetate (EA). The EA soluble containing a mixture of triterpenes were purified by silica gel column which yielded OA and MA which was recrystallised from chloroform and methanol. MA structure was confirmed by spectroscopic analysis using ^{1}H and ^{13}C Nuclear Magnetic Resonance techniques. Type 1 diabetes was induced by intraperitoneal injection of STZ (60mg/kgb.wt) which was prepared in citrate buffer. Control animals were injected with the citrate buffer. The effects of various MA doses on blood glucose were monitored in STZ-induced diabetic rats given a glucose load after an 18 h fast. Blood glucose was measured at 15 min interval for the first hour and hourly thereafter for 3 h. Blood glucose concentrations were also monitored in animals treated with MA for 5 weeks and these were measured after 6 h of treatment. Food and water intake, and weight were measured every third day at 9 and 24 h after treatment. All results were presented as mean± standard error means and $p<0.05$ was considered statistically significant. Acute oral glucose responses to MA indicate significant ($P<0.05$) blood glucose lowering effects in STZ-induced diabetic rats and antihyperglycaemic effects were exhibited after 5 weeks of treatment. Water intake was reduced, but food intake was not influenced. MA treatment significantly ($P<0.05$) increased body weight, this was beneficial as diabetic rats lose weight. Our results suggest that MA lowers blood glucose concentration suggesting that it can be a potential drug for management of type 1 diabetes mellitus.

Key words: Maslinic acid, plant extract, anti-hyperglycaemic effects, STZ-induced, diabetic rats.

The effect of C*yclopia maculata* on obesity in wistar rats

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Obesity is a major contributing factor to the global burden of chronic diseases such as type 2 diabetes. Studies have reported that bioactive compounds from plants have anti-obesity effects. This study investigated whether *Cyclopia*

maculata (fermented hot water extract) has anti-obesity properties in a Wistar rat model of diet-induced obesity. Non-obese and obese rats were fed a standard rodent or a high fat, high sugar cafeteria diet for 12 weeks, with or without *C. maculata* supplementation (300 mg/kg body weight). Orlistat (anti-obesity drug) served as a control. Body weights, food and water consumption and blood glucose levels were measured. After treatment, rats were terminated and retroperitoneal fat (RF) pads and liver weights were determined. The expression of key adipogenenic and adipolytic genes was quantified in RF pads using quantitative real time PCR. This study shows that *C. maculata* treatment inhibited weight gain by 12% in non-obese rats on a cafeteria diet. However, the same did not apply to obese rats. *C. maculata* treatment modestly (3.4%) inhibited weight gain in obese rats when they were fed a standard rodent diet. The failure of *C. maculata* to decrease weight gain was related to increased food intake. Blood glucose levels of obese rats were decreased at the end of the treatment period. The expression of Fasn and Pparα was decreased in the RF of cafeteria diet fed non-obese rats, while the expression of IL-6 was increased. No significant differences were seen in obese rats. This study suggests that *C. maculata* has the potential to be used as a weight reducing food supplement in obese individuals, only in combination with a healthy diet. These findings are in agreement with recommendations by commercially available weight loss supplements where adherence to a healthy diet is essential to optimal weight loss.

Key words: Obesity, diabetes, plant extracts, *Cyclopia maculate*, rats, diet supplements, weight gain.

Novel ways to blunt hyperglycemia-induced cardiac dysfunction

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Previous studies reported that higher blood glucose levels during and after an ischemic insult amplify the risk of future cardiovascular disease events. In agreement, we found that hyperglycemia triggers oxidative stress thereby resulting in greater flux of glucose through alternate metabolic pathways (e.g. the hexosamine biosynthetic pathway [HBP]), leading to increased myocardial apoptosis. Since benfotiamine (vitamin B-derivative) can decrease HBP flux, we hypothesized that it attenuates cardiac cell death and thus enhances heart function in response to ischemia-reperfusion under hyperglycemic conditions. We perfused isolated rat hearts with Krebs Henseleit buffer ±11, 22 and 33 mM glucose, respectively (Langendorff system) for 90 min, followed by 30 min global ischemia and 60 min reperfusion. To evaluate the cardioprotective effects of benfotiamine, 3 doses (25, 50, and 100 µM) were administered immediately after ischemia for the first 20 min of reperfusion. Our data show decreased ($p<0.05$) functional recovery of heart function under hyperglycemic conditions (15±4.3%) vs. matched controls (38±2.5%) following ischemia-reperfusion. However, benfotiamine treatment blunted the damaging effects of hyperglycemia and significantly ($p<0.05$) improved the heart's functional recovery. Our study demonstrates that benfotiamine is a promising cardioprotective agent that may ultimately benefit pre- and full-blown diabetic patients suffering from cardiovascular disease complications.

Key words: Hyperglycemia, cardiac dysfunction, benfotiamine.

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Is postoperative hypernociception associated with anxiety-like behaviour in rats?

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Existing animal models of postoperative pain have focused only on the sensory aspects of postoperative nociception, and have ignored the affective components of pain, such as anxiety, which in human studies have been shown to be important determinants of the overall pain experience and pain outcomes. Therefore we investigated whether anxiety-like behaviour was a feature of an established animal model of postoperative pain. Postoperative hypernociception was assessed on a daily basis for four days before surgery and nine days after surgery in ten male Sprague-Dawley rats that had undergone abdominal surgery. Nociceptive thresholds were tested using an anaesthesiometer, which was applied to the wound until the rats showed aversive responses. Anxiety-like behaviour was tested for in a separate group of fifty experimental and fifty control rats that had undergone the same surgical/sham intervention. The open field paradigm was used to test anxiety-like behaviour, and involved placing rats in a 1 m^2 arena and measuring their exploratory behaviour as exploratory behaviour is reduced in anxious rats. Surgery produced a significant decrease in nociceptive thresholds for up to six days after surgery; however there was no significant difference between control and surgery rats with regards to exploratory behaviour of a novel environment at any stage after surgery. Therefore rats do not display anxiety-like behaviour in the open field test after surgery, despite the presence of significant postoperative hypernociception.

Key words: Postoperative pain, anxiety-like behavior, open-field test.

An *in vitro* assessment of *Athrixia phylicoides* aqueous extract on glucose metabolism

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Athrixia phylicoides DC is an indigenous shrub found in the eastern parts of Southern Africa. A beverage derived from dried twigs and leaves is consumed by local communities for its medicinal properties. Several phenolic acids (including chlorogenic acid, 1,3-dicaffeoylquinic acid, other dicaffeoylquinic acids and other hydroxycinnamic acid derivatives) were identified that are linked to protection against oxidative stress as well as improving glucose metabolism. The aim of the study was to determine the *in vitro* effects of a hot water extract, prepared from the leaves and fine twigs of *A. phylicoides*, on cellular glucose metabolism in cell lines that mimic the three most influential tissues in maintaining glucose homeostasis (that is muscle, liver and fat). C2C12, Chang and 3T3-L1 cells were cultured under standard conditions. Glucose taken up by cells, acutely exposed to water vehicle and increasing concentrations of extract and the positive controls (insulin and metformin) was determined using a fluorimetric oxidase method. Radioactive ^{14}C-glucose oxidation to $^{14}CO_2$ and determination of glycogen content of cells was used to assess the metabolic fate of intracellular

glucose. RT-PCR was used to assess the extract effect on insulin-signalling gene expression. Glucose uptake in C2C12, Chang and 3T3-L1 cells was increased by 228.3% ($p<0.001$), 134.5% ($p<0.05$) and 143.5% ($p<0.001$), respectively. The oxidation of ^{14}C-glucose to $^{14}CO_2$ by C2C12 myocytes ($p<0.01$) and Chang ($p<0.05$) cells was increased following acute exposure to the extract. *A. phylicoides* extract increased glycogen storage in Chang cells compared to the control ($p<0.05$). Furthermore, an extract-induced increase in insulin receptor and glucose transporter four expression was seen in C2C12 myocytes (2.7 and 2 fold respectively). *A. phylicoide*s aqueous extract stimulated *in vitro* glucose uptake and metabolism, suggesting anti-diabetic potential.

Key words: Phenolic acids, oxidative stress, glucose metabolism, insulin receptor.

The influence of the menstrual cycle on sperm function

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The female menstrual cycle is earmarked by a complex interplay in changes in various hormonal concentrations and vaginal pH fluctuations. Despite the fact that spermatozoa spend their entire post-ejaculatory lifespan in the female reproductive tract, very little research has thus far been done on how these factors can influence spermatozoa. The aim of this study was therefore to investigate the effect of 8 different FSH, LH, progesterone and oestrogen concentrations as well as pH levels (i.e. representing menses, mid-follicular, late-follicular, LH & FSH surge, ovulation, early-luteal, mid-luteal, late-luteal phase) on sperm function. Spermatozoa were isolated from semen samples obtained from healthy donors according to the WHO criteria. Samples were subsequently exposed to the various concentrations of these hormones (alone or in combination) and different pH levels and incubated for 60 minutes at 37ºC. Afterwards sperm motility (CASA), viability (eosin/nigrosin) and acrosome intactness (FITC-PSA) were measured. Preliminary results obtained after progesterone and oestrogen exposure indicate that sperm motility and viability is reduced during menses and increases significantly until ovulation. Post-ovulation sperm function yet again starts to decrease significantly. This study therefore indicates that female reproductive hormones (1) affect sperm function and (2) in such a manner as to synchronize and optimize male gamete function to coincide with the female's fertility window in order to increase fertilization success.

Key words: Spermatozoa, female hormones, menstrual cycle, sperm motility, viability.

The relationship between semen viscosity and male genital tract infections

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A trend has been observed whereby an increasing number of patients attending the Reproductive Biology Unit at Tygerberg Hospital have been presenting with hyper viscous semen samples. A possible explanation for this phenome-

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non could be an increase of patients presenting with leukocytospermia, a condition defined by >1 × 10^6 WBC/ml. Studies on the cause of hyper viscosity and its relationship to altered sperm function and dysfunction of the male accessory sex glands would provide vital insight into a condition that has received little attention. The aim of this study was to determine whether a relationship exists between deviant viscosities due to male genital tract infection (MAGI). Seminal viscosity was measured and quantified by two methods; the filling time of a Leja semen analysis chamber converted to centipoise (cP) and the subjective assessment of the length (cm) of semen released from a wide-bore pipette. The possible presence of MAGI was assessed biochemically by an enzyme immunoassay (PMN Elastase), as well as histochemically by a leukocyte peroxidase test. The glandular functioning of the prostate and seminal vesicles was assessed by the photometric quantification of the secretory products of citric acid and fructose respectively. Hyperviscosity was not associated with glandular function of the prostate or the seminal vesicles. A significant correlation was found between leukocytospermia and an increase in seminal viscosity ($r = 0.314$; $P<0.0001$). There was also a positive correlation between peroxidase positive cells and the PMN elastase concentration. Increased seminal viscosity may be an indication of MAGI and should be regarded as an indication for further examinations and treatment of the male patient as it is known that the presence of leukocytospermia can be associated with poor fertilization rates and low pregnancy outcome in ART.

Key words: Semen, viscosity, leukocytomspermia, male, genital tract, infection.

The hexosamine biosynthetic pathway induces gene promoter activity of the cardiac-enriched isoform of acetyl-CoA carboxylase

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The cardiac isoform of acetyl-CoA carboxylase (ACCβ) produces malonyl-CoA, a potent inhibitor of mitochondrial fatty acid (FA) uptake. Higher ACCβ activity decreases FA utilization, potentially leading to intracellular myocardial lipid accumulation and insulin resistance (IR). Since increased hexosamine biosynthetic pathway (HBP) flux is linked to IR onset, we hypothesized that HBP-mediated induction of ACCβ gene expression is a significant contributor to IR. We transiently transfected cardiac-derived rat H9c2 myoblasts with a 1317 bp human ACCβ promoter-luciferase construct (pPIIβ-1317) ± a GFAT (HBP rate-limiting enzyme) expression construct ± HBP modulators. Here administration of L-glutamine (HBP substrate) dose-dependently increased, while GFAT inhibitors attenuated pPIIβ-1317 activity. Co-transfections with a dominant-negative GFAT construct also diminished pPIIβ-1317 activity. To explore underlying transcriptional mechanisms, we investigated upstream stimulatory factors (USF) and found that USF2 induced pPIIβ-1317 activity vs. controls. Moreover, co-transfection of a GFAT expression construct + USF reporter-promoter construct (with consensus USF binding elements) lead to a marked induction vs. controls. We next performed transfections with GFAT ± the full length ACCβ and 4 truncated promoter-luciferase constructs, respectively. GFAT overexpression increased ACCβ promoter activity for the full length and 3 larger deletions constructs. However, GFAT-mediated ACCβ promoter induction was blunted when co-transfected with the -38/+65 deletion construct indicating that USF2 binds to the proximal promoter region (near start codon). Our study demonstrates that increased HBP flux induces ACCβ gene promoter activity via modulation of USF2 promoter binding. We propose that ACCβ induction reduces FA oxidation, thereby contributing to the onset of cardiac IR.

Key words: Acetyl-CoA carboxylase, hexosamine biosynthetic pathway, insulin resistance.

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The impact of circulating cholesterol concentrations on carotid intima media thickness in an urban, developing community of African ancestry

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Cholesterol is an important determinant of atherogenesis. However, populations of African ancestry have low total and LDL cholesterol concentrations (anti-atherogenic), but elevated triglyceride and low HDL concentrations (pro-atherogenic). The role of circulating lipids in atheroma formation in groups of African descent is uncertain. Therefore, the aim of the current study was to determine whether circulating lipids are independently associated with carotid intima media thickness (C-IMT), a surrogate marker of atheroma, in an urban developing community of African ancestry in SOWETO South Africa. 446 participants were randomly selected. C-IMT was determined from Doppler images of the carotid artery using a SonoSite (SonoCalc^{TM} IMT) version 3.4 device. In univariate analysis total cholesterol, total: HDL cholesterol ratio, HDL cholesterol, LDL cholesterol and triglyceride concentrations were associated with C-IMT ($r = 0.11$ to $r = 0.21$, $p<0.005$). However, in multivariate models which included adjustments for age, conventional systolic blood pressure, diabetes mellitus, smoking, treatment for hypertension, regular alcohol intake, heart rate and postmenopausal status (confirmed with follicle stimulating hormone measurement); none of the lipid variables were markedly associated with C-IMT (total cholesterol $p = 0.05$, total:HDL cholesterol ratio $p = 0.60$, HDL $p = 0.60$, LDL $p = 0.29$, triglycerides $p = 0.33$). In conclusion, in urban developing communities of African ancestry no major independent relationship between circulating lipid concentrations as indicated by C-IMT and atheroma formation occur.

Key words: Cholesterol, atherosclerosis, conventional blood pressure.

Amino acid starvation induced autophagy during doxorubicin treatment in breast cancer cells

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Growing tumours develop regions with limited access to nutrients, due to spatial separation from the native vascular bed. However, it appears as though some cancers may have the potential to maintain intracellular amino acid levels over short periods through the endogenous promotion of macroautophagy. Some current therapeutic strategies target autophagy with the objective of countering this survival mechanism. We investigate amino acid induced autophagy during doxorubicin treatment and pose the contrasting hypothesis that augmented autophagy following amino acid withdrawal will correlate with an increased sensitivity of breast cancer to doxorubicin. Breast cancer and epithelial cells were cultured in typical growth medium or medium without amino acids. Steady state autophagy levels were monitored using protein markers (LC3-II and beclin-1) and the degree of acidic compartmentalization in cells, while mass spectrometry based proteomics was used to analyse cellular amino acid levels. Cell death was analysed morphologically and

through examination of apoptosis markers. Mouse tumours sizes were examined using standard measurement techniques. Amino acid deprivation results in dynamic, and cell specific, time dependent changes in apparent autophagy. We suggest that this acts to preserve amino acid balance within the cell. Our evidence indicates that amino acid withdrawal results in elevated autophagy when in association with the anti-tumour drug, and although we present evidence that this also results in increased apoptosis and decreased tumour volume we argue against this as the only mechanism for increased doxorubicin efficacy following sensitization. This novel approach to tumour sensitization could have several implications in the context of cancer therapy, and given the delicate relationship that autophagy has with the cancer microenvironment, efforts to determine the mechanisms involved in autophagy and sensitization could lead to new and innovative treatment opportunities for cancer management.

Key words: Breast cancer, autophagy, Doxorubicin, LC3.

FTY720: To activate or not to activate – that is the question?

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Recent studies have underscored the physiological importance of the serine/threonine protein phosphatase, PP2A-c. Cardiovascular studies focused mainly on the inhibition of PP2A-c, which elicited cardioprotection following ischaemia/reperfusion injury (IRI). The sphingosine-1-phosphate agonist, FTY720, has been shown to activate PP2A-c and has recently been approved for human use. Few studies have been done on the effects of FTY720 in the setting of myocardial IRI. Our aim was to investigate the effect of FTY720 on the phosphorylation states of PP2A-c and the kinases involved in the Reperfusion Injury Signalling Kinase (RISK) pathways of the heart, under baseline conditions. Isolated rat hearts were perfused using the work heart model. Stabilization was followed by a 15 min administration of three different concentrations of FTY720 (0.5, 1.0 and 2.5 μM) and the hearts freeze clamped directly after administration. Collected tissue was analysed using standard Western blotting techniques. Coronary flow was measured throughout the administration period. The coronary flow increased with increasing dosages of the drug (control: 11.29±0.01 vs. 2.5 μM FTY720: 21.16ml/min±0.804 ml/min, n = 2-4; $p<0.05$), suggesting a dilatory effect. Lower concentrations of FTY720 was associated with a reduction in the phosphorylation (therefore increased activity) of PP2A-c (control: 30640±4023 vs. 0.5μM: 19680±2577 and 1.0μM: 17970±1454 pixels, n = 2-4; $p<0.05$). In accordance with these results, 2.5μM FTY720 was associated with an increased phosphorylation of PKB (2.5 μM: 13680±1061 vs. 0.5 μM: 9310±545 and 1.0 μM: 9495±443.2, n = 3-4; $p<0.05$) and combined ERK p42/p44 (ERK p42/p44: 2.5 μM: 3164±328.2 vs. 1.0 μM: 1885±67.93 pixels, n = 3-4; $p<0.05$). In conclusion, FTY720 shows dose-dependent effects in the heart. At low (0.5 and 1.0 μM) concentrations FTY720 potentially activates PP2A-c, while at 2.5 μM FTY720 exerted a vasodilatory effect, as well as enhanced phosphorylation of the RISK pathway. Future work will focus on the effects of FTY720 on insulin signalling in the heart.

Key words: Protein phosphatase, myocardial ischemia-reperfusion injury, RISK pathway.

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Scientific Research and Essays

Expression profile of Wnt isoforms during differentiation of aging myoblasts

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Satellite cells are muscle progenitor cells responsible for skeletal muscle maintenance and repair. The myogenic capacity of these progenitor cells decreases with age causing muscle repair to be characterised by fibrosis and lipid accumulation. Recent studies have suggested that the Wnt family of signaling proteins is pivotal in regulating cell fate during aging. In the current study, we aim to determine the cytosolic expression and the secreted profiles of Wnt3a, 7 and 10b, during myogenesis of early- and late-passage C2C12 myoblasts. Differentiating early- (P15) and late-passage (P33) murine C2C12 myoblasts were harvested at days 0, 1, 3, 5 and 7, and conditioned media collected at days 0, 1, 3 and 5. Conditioned media was concentrated to 500 µl with PEG 20 000. Cytosolic and secreted protein expression was determined by Western Blotting. When compared with early-passage cells, late-passage cells were characterized by a higher proliferative rate and decreased terminal myogenic differentiation. Analysis of Wnt7 and 10b revealed higher baseline expression in proliferating late-passage cells when compared with the early-passage counterparts. However, in response to differentiation cues, late-passage cells displayed a more rapid decline in Wnt7 expression and secretion. Wnt10b expression in late-passage cells decreased rapidly in response to differentiation media, whereas early-passage cells maintained steady expression. Wnt3a expression did not differ substantially between early- and late-passage cells. Finally, in response to differentiation, the ratio of secreted to cytosolic (S:C) Wnt protein levels were lower for Wnt7 and Wnt10b, but not Wnt3a in late- versus early-passage cells. Taken together, these results suggest that both Wnt7 and Wnt10b are important for successful myogenesis. Studies using recombinant proteins are underway.

Key words: Signalling proteins, myogenesis, differentiation, Wnt proteins.

In vitro comparative analysis of street methamphetamine ('tik') cytotoxicity on mouse brain endothelial (bEnd5) cells

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Research conducted in the Western Cape, Cape Town, South Africa showed that the average age of patients, who reported street methamphetamine (MA), commonly known as 'tik' as their primary substance of abuse, in the first half of 2008 were 23 years where 74% were reported to be male. 'Tik' is an illegal highly-addictive drug, with structural similarities to neural monoamines such as dopamine and serotonin. Studies demonstrated that abuse, leads to a compromised blood-brain-barrier essential to the homeostasis of the central nervous system. This study attempted to elucidate and compare the cytotoxic effects of pure and street MA on mouse brain endothelial (bEnd5) cells, which to date, has not yet been published. Cytotoxic effects of selected concentrations of pure and street MA at various time intervals were analysed on bEnd5 cells using Trypan Blue Viability and LDH assays. Statistical analysis was performed using the Wilcoxon Rank Sum Test. $P < 0.05$ was denoted as significant. Notable increase in cell growth of 177-fold ($P =$

0.0209) was observed between 24 and 96 hours in controls, while pure and street MA-exposed cells displayed maximum growth of 134.5 fold (P≤0.0209), and 110-fold (P≤0.0139), respectively. Cells exposed to pure and street MA displayed biphasic activity for all time intervals. Both experimental groups showed significantly lower cell numbers than controls at 96 hours, while street MA resulted in markedly decreased cell growth compared to pure MA. Significant growth differences were observed between control and experimental groups, however no toxicity was observed, (supported by percentage viability of 93.87 ± 3.07 for MA-exposed cells). Pure and street MA exposure resulted in significant cell growth inhibition without affecting viability. Inhibitory effects were more pronounced in street meth-exposed cells compared to pure MA-exposed and control cells. Impurities may be responsible for the exacerbated adverse effects of street MA.

Key words: Methamphetamine, mouse brain endothelial (bEnd5) cells, cytotoxic effects.

Caffeine induces hyper-acetylation of histones at the MEF2A binding site on the Glut4 promoter via p300 upregulation

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The expression of GLUT4, the major glucose transporter in skeletal muscle, is increased by caffeine and regulated by myocyte enhance factor-2A (MEF2A) in C2C12 myotubes. Caffeine-induced GLUT4 expression is associated with hyperacetylation of histones surrounding the MEF2A binding site on the Glut4 gene but the histone acetyl-transferases (HATs) involved are unknown. Acetylation of histones at gene promoters increases the accessibility of transcription factors to their respective promoters to enhance transcriptional activity. This study aimed to determine whether p300, an ubiquitous HAT known to interact with MEF2 transcription factors, is involved in acetylating histones at the Glut4 gene. Differentiated C2C12 myotubes were incubated with 5mM caffeine and/or 25 µM or 40 µM Curcumin, a potent inhibitor of p300 HAT activity, for 1hr. Chromatin Immunoprecipitation (ChIP) assays were used to measure the levels of acetylated histone H3 (Lys 9/14) and the amount of MEF2A bound to the Glut4 promoter. Western Blotting was conducted to determine total acetyl H3, p300 and GLUT4 contents. Caffeine treatment increased total acetylated H3 content and p300 (HAT) ~ 2-fold relative to controls. A similar increase was observed in acetylated H3 surrounding the MEF2A binding site. When Curcumin was co-treated with 5 mM Caffeine, p300 levels were reduced in a dose dependent manner: 40 µM Curcumin blocked the increase in total p300 and total acetyl H3 and acetyl H3 at the Glut4 gene. The increase in GLUT4 induced by caffeine treatment was effectively inhibited by 25 µM Curcumin. Caffeine-induced hyper-acetylation of the MEF2A binding domain on the Glut4 promoter and GLUT4 expression appears to involve p300.

Key words: GLUT4 expression, caffeine, MEF2A binding domain.

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Scientific Research and Essays

Anthracycline-induced-cardiotoxicity: Role of proteolytic pathways

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Cardiotoxicity is a major hurdle limiting the use of Doxorubicin (DXR), the most effective and extensively used anticancer agent of the anthracycline family. Although the precise mechanism by which DXR damages the heart remains to be fully elucidated, free radical-induced oxidative stress from DXR metabolites play a fundamental role. Antioxidant therapy however has not been able to entirely eliminate cardiotoxicity thus indicating that DXR-induced cardiotoxicity is multifaceted and complex. Autophagy, an intralysosomal degradation of the cells' own constituents, is used as an important survival mechanism in the presence of external stressors, intracellular stimuli and provides protection against diverse pathologies including heart disease. We thus aimed to determine whether elevated autophagy would prove beneficial during chemotherapeutic treatment. Elevated autophagy was induced using rapamycin (50 nM) for 24 h in H_9C_2 myoblasts where after DXR (3 µM) was added for an additional 24 h. Mitochondrial viability and cell death were assessed using various assays. Furthermore, mitochondrial and endoplasmic reticulum morphology, ubiquitin proteasome activity, ROS production and calcium handling was assessed using fluorescence microscopy and flow cytometry. Significant reductions in mitochondrial viability were observed in the DXR treatment group ($p<0.01$, 65.58±2.47%) whereas the combination of rapamycin and DXR produced significant improvement in viability ($p<0.05$, 78.93±10.85%). Cell death through apoptosis increased during DXR treatment ($p<0.05$, 216.1±33.51%), but was attenuated when autophagy was upregulated in the presence of DXR ($p<0.05$, 31.97±13.92%). ROS analysis proved that mitochondria are the source of ROS production during DXR treatment though elevated autophagy was unable to significantly reduce mitochondrial ROS. We have demonstrated that increased autophagy is a vital survival mechanism in H_9C_2 myoblasts during acute DXR treatment. This evidence can provide novel treatment strategies for patients who developed anthracycline-induced cardiotoxicity.

Key words: Anthracycline-induced cardiotoxicity, proteolytic pathways, myoblasts.

Bio-artificial muscle development and implementation for a severe injury repair model

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Bio-artificial muscles (BaMs) are grown within a 3-dimensional scaffold between two anchor points which act as tendon attachment sites. These muscle constructs have the ability to contract under electrical stimulation and mimic *in vivo* muscle in terms of tubule formation and contractile force generation. Predominantly immortalized cell lines are used to grow BaMs, such as the murine C_2C_{12} cell line; however, this leads to problems in translating results for clinical trial applications. Due to this, primary cultured murine and human skeletal myoblasts would be more appropriate for BaM formation. Primary culture for myoblast isolation is a difficult process due to fibroblast contamination. We have therefore optimized an isolation protocol using several pre-plating techniques to obtain a >95% pure myoblast population for

expansion. The BaM formation protocol utilizes extracellular matrix scaffolds and electrical stimulation to aid in correct tubule formation during fusion of the myotubes. To date we have optimized our primary isolation protocol allowing the use of directly isolated myoblasts to form our *in vitro* tissue. We have also optimized the gel scaffold and tissue formation (utilizing various ECM combinations including Matrigel, collagen I and fibrin) allowing us to achieve complete attachment of the gel/cell suspension across the two anchor points. Currently, we are optimizing our electrical stimulation protocol to enhance myotube alignment and subsequent force production capability of the BaMs.

Key words: Myotube, bio-artificial muscles, extracellular matrix scaffolds.

Muscle atrophy: Is long-term antiretroviral treatment a causative agent?

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Successful antiretroviral (ARV) treatment is associated with the suppression of HIV viral load and the reduction of clinical disease progression. However, despite marked improvements, side effects from long-term therapies, such as loss of muscle mass do occur. The mechanism by which ARVs affect muscle mass is unclear, however, published *in vitro* data indicates an effect on myoblast fusion during differentiation. The objective of this study is therefore to determine the effect of ARVs on other processes required for successful myogenesis, such as proliferation and migration. Zidovudine (nucleoside reverse transcriptase inhibitor-NRTI), Tenofovir (nucleotide reverse transcriptase inhibitor-NtRTI) and Ritonavir (protease inhibitor-PI) were utilized at a concentration range of 0.01 to 10 µM. C2C12 cells were used as the model myoblast cell line. Proliferation was determined using crystal violet, whereas migration was analyzed using 2D wound healing assay. Short-term incubation with all three ARVs had no significant effect on proliferation or migration. However, following long-term incubation, proliferation was increased in response to all three ARVs. Furthermore, migration was observed to have increased in response to Zidovudine and Tenofovir, but not Ritonavir. To our knowledge, this study is the first to suggest that chronic incubation with selected ARVs may significantly influence myoblast proliferation and migration. An increase in these processes may result in a depletion of the satellite cell population, thereby contributing to a decrease in muscle mass. Studies to analyze the self-renewal capability of myoblasts in response to ARVs are underway.

Key words: Muscle atrophy, antiretroviral treatment, myoblast proliferation.

Exposure to early life stressors enhances the prevalence of febrile seizures in young rats

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A febrile seizure is neurological disorder that occurs following an infection that results in a rapid rise in body temperature. This disorder commonly affects children between the ages of 3 months and 5 years. Existing evidence suggests that neurological disorders can be exacerbated in offspring exposed to stress prenatally. Currently there is no cure for febrile seizures but epileptic drugs such as valporate and benzodiazapem are used for the symptomatic alleviation of seizures. These treatments however do not to prevent the occurrence of the seizure nor its reoccurrence. Studies have shown that traditional plants such as *Rhus chirindensis* (rhus) may have anti-epileptic properties. In our study we investigated whether febrile seizures are exacerbated in the offspring of rats that were prenatally stressed and whether *Rhus* can prevent the recurrence of febrile seizures. To induce a seizure we used lipopolysaccharide followed by Kainic acid. Behaviour was assessed by using a light dark box which measures anxiety like behaviour. Our results show that 1). Stress exacerbates anxiety like behaviour 2). Stress prolongs the duration of the seizure and 3). *R. chirindensis* also shows to have antagonistic effects against lipopolysaccharide and kainic acid.

Key words: Febrile seizure, prenatal stress, behaviour, traditional plants, kainic acid.

The relevance of the washout episode in β-preconditioning

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The heart can be protected against ischaemic damage by exposure to either 5 min global ischaemia (ischaemic preconditioning, IPC) or administration of a β2-adrenergic agonist, formoterol (β-preconditioning, β2-PC), followed by a 5 min washout period before the heart is subjected to sustained ischaemia. The washout period is essential for cardioprotection, probably due activation of certain signal transduction pathways. The aim of the study was thus to characterize the signaling pathways which are activated during the washout period after IPC or β2-PC. Male Wistar rats (±240 g) were used. Hearts were rapidly excised, arrested in ice-cold buffer, mounted onto the perfusion rig and retrogradely perfused. After a stabilization period of 40 min, IPC hearts were exposed to 5 min global ischaemia, followed by 5 min reperfusion. β2-PC hearts were perfused with formoterol hemifumarate (1 nM) for 5 min, followed by 5 min washout. Hearts were freeze-clamped at 0, 1, 3 and 5 min during washout. Non-preconditioned hearts (NPC) were untreated and freeze-clamped after similar perfusion time periods. N = 6 hearts/group. Western blots were done for PKB, ERK, JNK and p38MAPK. Results are expressed as means±SEM. The 3 and 5 min washout periods after β2-PC showed similar significant activation of ERKp44 (3.782±0.52 vs. 1.409±0.34; $p<0.05$) and ERKp42(2.195±0.32 vs. 1.179±0.12; $p<0.05$) compared to 0 and 1min washout episodes. PKB similarly showed significant activation (4.119±0.52 vs. 1.574±0.12; $p<0.001$) 5min into the washout period. Analysis of data obtained thus far indicates a significant activation of kinases known to be associated with cardioprotection (PKB and ERK, the so-called RISK pathway) to occur during a preconditioning protocol. Thus the data obtained suggest that cardioprotection is associated with activation of the RISK pathway during both the preconditioning protocol as well as during reperfusion.

Key words: Preconditioning, RISK pathway, reperfusion.

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Scientific Research and Essays

Acute exercise decreases central pressures in people with mild to moderate hypertension

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Exercise is beneficial in the management of hypertension as post exercise hypotension [PEH, a reduction in resting blood pressure (BP) after acute exercise] occurs. PEH has only been demonstrated using measurements of peripheral (brachial) BP; however, central (aortic) BP more accurately predicts myocardial workload and is a better prognostic marker of cardiovascular mortality and morbidity. We determined the central and brachial BP response to acute aerobic exercise of moderate intensity in mild to moderate hypertensive subjects. Currently, eleven men and women (age 30 to 57 years) with untreated pre-hypertension (systolic BP, SBP 120 to 139 mmHg or diastolic, DBP 80 to 89 mmHg) or stage I hypertension (SBP 140 to 159 mmHg or DBP 90 to 99 mmHg) have volunteered for the study. Brachial BP, and central BP and pulse wave analysis (applanation tonometry, SphygmoCor software) were determined at rest (day 1), then at 15 min and 24 h after an acute exercise session (day 2). Brachial ambulatory BP was also monitored for 24 h on both days. Repeated measures ANOVA showed that compared to baseline, peripheral SBP, DBP and pulse pressure (PP), and central DBP and pulse wave velocity were no different at 15 minutes or 24 h after exercise ($p>0.05$). However, significant decreases in central SBP (mean±SD; 8.5±11.0 mmHg; $p = 0.03$), central PP (6.4±8.4 mmHg, $p = 0.04$) and augmentation pressure (9.0±3.8 mmHg ($p<0.0001$) occurred at 15 minutes after exercise. The decrease in central SBP tended to remain decreased 24 h after exercise (6.9 ± 12.1 mmHg; $p = 0.09$); but central PP and augmentation pressure were no different from baseline. In conclusion, acute exercise produced decreases in central pressures that were not observed peripherally. The change in central SBP is likely to be attributed to a change in the properties of medium sized arteries, and not an acute change in arterial stiffness.

Key words: Hypertension, exercise, blood pressure.

Effect of JNK inhibition on functional recovery and kinase patterns during reperfusion of hearts from obese insulin resistant rats

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Insulin resistance can decrease glucose uptake, alter lipid metabolism and impair PKB-dependent signalling in its target tissues. PKB activation is also central to myocardial survival during ischaemia/reperfusion (I/R). It has recently been suggested that activation of the stress kinase C-jun-N-terminal kinase (JNK) is essential for PKB phosphorylation at the onset of reperfusion. Previous studies from our lab also indicated an important role for JNK in I/R. We hypothesize that inhibition of JNK will affect functional recovery and interactions between JNK, PKB, p38 and ERK activation during reperfusion in hearts from obesity-induced insulin resistant rats. Insulin resistance was induced by feeding rats a high calorie diet for 16 weeks (DIO). Hearts from DIO and age-matched controls (C) were pretreated with the specific JNK inhibitor, SP600125 (SP; 10μM), for 10 min before 15 min sustained global ischaemia followed by different reperfusion

times to determine functional recovery and the kinase activation pattern (Western blotting). Substrates were glucose (10mM) plus fatty acid (1.2 mM palmitate /3%BSA). N = 3-5/group. Inhibition of JNK by SP had profound effects on the recovery pattern of the hearts from both DIO and control rats: functional recovery was significantly reduced by 54% and 34% respectively (% recovery of total work: D+SP/D 51.1±6.3/105.4±3.6, p< 0.05; C+SP/C 59.1±8.6/92.7±3.9, p< 0.05), associated with increased activation of p38 and inactivation of ERKp42 and PKBs473 at different time intervals (D+SP/C+SP 5' p38 1.26±0.05/1.00±0.01, ERKp44 1.47±0.04/1.00±0.14, ERKp42 1.45±0.07/1.00±0.13; p<0.05, respectively; D+SP/D p38 5': 1.61±0.06/1.00±0.08, 10': 2.81±0.39/1.00±0.21; p<0.05, respectively; C+SP/C 10' p38: 1.70±0.17/1.00±0.22, ERKp42: 0.66±0.02/1.00±0.06, 30' PKBs: 0.44±0.01/1.00±0.18; p<0.05, respectively). However, JNK phosphorylation did not change during reperfusion. In conclusion, inhibition of JNK activation during I/R has detrimental effects on functional recovery, with hearts from DIO animals being more susceptible. The reduction in functional recovery was associated with time-dependent increased activation of p38 MAPK (D>C) and inactivation of ERK (D>C) and PKB.

Key words: Insulin resistance, ischaemia/reperfusion, MAPK.

Tumour necrosis factor (TNF)-α and hypoxia: towards a model of endothelial dysfunction

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The endothelium carries out a diverse array of functions. Due to its juxtaposition with the lumen, vascular endothelial cells are the first line of defense against numerous deleterious stimuli (e.g. hypoxia and TNF-α). If these are sustained, progression to atherosclerosis and ischaemic heart disease can follow. In this study, we aimed to establish a model of endothelial hypoxia, and compare hypoxic responses (apoptosis, necrosis and nitric oxide (NO) production) with those observed in TNF-α-stimulated endothelial cells. Cardiac microvascular endothelial cells (CMECs) were subjected to the following experimental interventions: (i) 0% serum growth medium without supplements ("modified medium") ± hypoxia for 6, 24 and 48 h; (ii)10% serum growth medium with supplements ("normal medium") ± hypoxia for 6, 24 and 48 h; and (iii) 5 ng/ml TNF-α treatment for 24 h. Hypoxia was induced by <1% O_2 incubation. Parameters measured: Apoptosis (AlexaFluor 647-AnnexinV conjugate-fluorescence), necrosis (propidium iodide-fluorescence) and intracellular NO-production (DAF-2/DA-fluorescence). Our results shows: Hypoxia 6 h: apoptosis 52% and 57% of control (normal and modified medium; p<0.05); no significant necrosis change; 24 h: apoptosis 243.69% and 142.16% (p<0.05), necrosis 199% (normal medium; p<0.05); 48 h: apoptosis 141.56% and 122.88% (p<0.05). NO-production increased significantly after 24 and 48 h hypoxia, especially with modified medium. Treatment with TNF-α (24 h) increased DAF fluorescence (+12.2% ± 1.9%), while apoptosis and necrosis were significantly lower (-33.04 ± 5.7 and -9.73 ± 11.8 respectively). Our 6h hypoxia results support previous findings that endothelial cells are relatively resistant to hypoxia up to a point. The protective responses disappeared at 24 and 48 h hypoxia. Both hypoxia (24 and 48 h) and TNF-α-treatment (24 h) resulted in significantly increased NO-production; however TNF-α-treatment was associated with an overall pro-survival response, which was not observed in the hypoxia groups. It would be interesting to investigate whether these trends will be sustained in the 48 h groups.

Key words: Endothelium, nitric oxide, tumour necrosis factor, apoptosis, necrosis.