AN ASSOCIATION BETWEEN NON ALCOHOLIC STEATOHEPATITIS AND POLYCYSTIC OVARIAN SYNDROME

M. M. Brzozowska, R. Coles, K. Park, M. Weltman

Endocrinology/Gastroenterology, Nepean Hospital, Kingswood, NSW, Australia

OBJECTIVES: The aim of this study was to determine if there is an association between non-alcoholic steatohepatitis (NASH) and polycystic ovarian syndrome (PCOS). NASH and PCOS are known to be associated with metabolic syndrome/insulin resistance. METHOD: The study recruited 14 consecutive female patients of reproductive age (20-45) either with liver biopsy proven NASH (50%) or positive abdominal ultrasound US (50%). Diagnosis of PCOS was defined by criteria from 2003 Rotterdam consensus meeting. Other causes of hyperandrogenism were excluded. All subjects underwent relevant questionnaire and clinical exam, had appropriate serum hormonal assays, pelvic (1) or transvaginal US (13) and were screened for evidence of the metabolic syndrome.

RESULTS:
Eleven subjects (79%) had clinical evidence of PCOS with oligo/amenorrhoea (8), hyperandrogenism (9), and infertility (6). Seven women (50%) had evidence of biochemical hyperandrogenism with low SHBG, raised free testosterone and elevation of serum LH concentration. Seven subjects (50%) fulfilled US criteria for PCOS. Overall ten out of fourteen subjects matched diagnostic criteria for PCOS (71%). CONCLUSION: Despite limitations of the study due to the sample size we found evidence of PCOS in the majority of subjects with NASH. The diabetic patients were in PCOS group (3/10). IMPLICATIONS: Women with NASH should be routinely screened for presence of PCOS, Diabetes Mellitus and metabolic risk factors for cardiovascular disease. Equally women with PCOS should be screened for NASH. The serum fasting insulin level was not helpful in discriminating between women with or without PCOS.

SRB ORALS FOR JOINT SESSIONS

EXPRESSION OF COMPONENTS OF THE HEDGEHOG SIGNALLING PATHWAY DURING MURINE SPERMATOGENESIS

A. Szczepny1,2, D. A. Jans2,3, G. Hime2,4, K. L. Loveland1,2

1Monash Institute of Medical Research, Monash University, Clayton, VIC, Australia
2ARC Centre of Excellence in Biotechnology & Development, Australia
3Department of Biochemistry & Molecular Biology, Monash University, Clayton, VIC, Australia
4Department of Anatomy & Cell Biology, Melbourne University, Parkville, VIC, Australia

Hedgehog (Hh) signalling is best known for its involvement in regulating patterning, driving cell proliferation, promoting cell survival and directing differentiation during embryonic development (1). The role that Hedgehog signalling plays in the testis is not yet clearly defined, though deletion of one Hedgehog ligand, Dhh, leads to male infertility. The Gli family of zinc finger TFs, consisting of Gli1, Gli2 and Gli3, are mediators of the Hh signalling cascade in vertebrates. We have previously shown that the mRNA transcripts encoding all three Gliis in the adult mouse testis are expressed highly in spermatogonia, spermatocytes and to a lower extent in the round spermatids. To understand the potential sites of action of Hh proteins in spermatogenesis, we have extended our analysis to other genes involved in the Hh signalling pathway in the adult mouse testis. Using in situ hybridization, Patched2, a transmembrane receptor for Hh, was detected in spermatogonia and spermatocytes, with an apparently lower expression in the round spermatids. The mRNA of Smoothened, another transmembrane protein which forms a membrane receptor complex with Patched, is highly expressed in spermatogonia and spermatocytes, again showing lower expression in round spermatids and interstitial cells. Fused, a positive regulator of Hh signalling, is highly expressed in spermatogonia and spermatocytes with slightly lower expression in round spermatids. SuFu is a negative regulator of Hh signalling, known to repress Gli1 function in part by tethering it in the cytoplasm. The mRNA encoding SuFu is absent from spermatogonia, detected in spermatocytes and persists in round spermatids where its expression appears highest, suggesting that the SuFu protein may be acting to switch off Hh signalling at that stage of spermatogenesis. Overall, the regulated expression pattern of these genes in the adult mouse testis suggests a role for Hh signalling in the regulation of spermatogenesis.

OESTROGEN RECEPTOR BETA IS INVOLVED IN THE REGULATION OF LEYDIG CELL NUMBER IN THE MOUSE.

M. Gould, H. D. Nicholson

Department of Anatomy & Structural Biology, University of Otago, Dunedin, New Zealand

Recent evidence suggests that oestrogen plays a physiological role in the testis. Both oestrogen receptor alpha and oestrogen receptor beta (ERβ) are present in the testis and administration of oestrogen has been shown to inhibit the development of Sertoli, Leydig and germ cells. This study investigates the effect of ERβ on the testis using ERβ knockout mice (bERKO). Adult male bERKO mice (n=8) and their wild-type littersmates (n=7) were killed at 11 weeks postpartum. One testis from each animal was fixed in Bouin's fluid and embedded. Each testis was fractionated and thick sections cut and stained with PAS. The optical dissector method was used to count the number of Leydig cells, Sertoli cells, spermatagonia, spermatocytes and spermatids in each testis. Trunk blood was collected and plasma testosterone concentrations measured by radioimmunoassay. No significant differences in body or testis weight were seen between the bERKO or wild-type mice. Similar numbers of Sertoli cells, spermatagonia, spermatocytes and spermatids were also observed between the two groups. The number of Leydig cells was significantly increased in bERKO mice compared with their wild-type littersmates (P< 0.05). Despite the increased number of Leydig cells in the bERKO mice there was no significant difference in plasma testosterone concentrations in this group compared to the wild-type mice. Oestrogen has been reported to inhibit proliferation of adult-type Leydig cells and to inhibit steroidogenesis. This study suggests that the regulation of Leydig cell proliferation may be mediated by ERβ. The presence of normal circulating testosterone concentrations in bERKO mice suggests that the effects of oestrogen on steroidogenesis are not brought about by ERβ.

FIBROBLAST GROWTH FACTOR RECEPTOR-1 (FGFR-1) IS ESSENTIAL FOR SPERMIOGENESIS, CAPACITATION AND MALE FERTILITY

L. M. Cotton1, G. M. Gibbs2, D. M. De Kretser1,2, M. K. O’Bryan1,2

1 Monash University, Monash Institute of Medical Research, Clayton, VIC, Australia
2 ARC Centre of Excellence in Biotechnology and Development, Australia

Male infertility is often a result of irregular sperm development/function. The identification of snt-2 (Suc-1 associated Neurotrophic Factor Target 2) and Fgfr-1 to the sperm tail, lead to the hypothesis that Fgf signalling through snt-2 is involved in sperm tail development/function. To test this hypothesis, transgenic mice carrying a dominant-negative variant of Fgfr-1, driven by the protamine 1 promoter (haploid specific) were created. Breeding experiments confirmed male fertility, however one line was significantly sub-fertile and demonstrated a significantly reduced daily sperm production, (DSP, 30%). Transgene expression levels were up to 70 times above native mRNA levels in wt mice; however there was a concurrent up-regulation of the native receptor in transgenic mice, resulting in only a 6x over-expression in transgenic: native mRNA. To increase transgene expression, independent lines were crossed (double heterozygous, DH). DH transgene expression levels were up to 120 times above the native mRNA in wild type mice, resulting in a 20x over-expression in transgenic : native mRNA. Breeding experiments showed males from 1 cross were significantly subfertile with DSPs further reduced, (41%). Collectively this data shows Fgfr-1 signalling is required for quantitatively normal spermogenesis. Given the millions of sperm that mice produce, a 40% in DSP is unlikely to be responsible for the sub-fertility observed ie. 2 versus 9 pups/litter. Therefore, a disruption of Fgfr-1 signalling may also induce a post-testicular phenotype. Western blot analysis, using tyrosine phosphorylation as a surrogate marker of sperm capacitation, showed transgenic mice had a significantly attenuated ability to initiate capacitation. As capacitation is an absolute requirement for fertilisation, the absence of capacitating capability is probably the major contributor to the sub-fertility seen in the transgenic mice. This research demonstrates for the first time that the Fgfr-1 signalling cascade is one of several pathways associated with sperm development and function.
ADULT EXPOSURE TO DIETARY PHYTOESTROGENS REDUCES FERTILITY OF MALE RATS

A. Glover, S. J. Assinder

Andrology Research Group of Otago, Department of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand

Phytoestrogens are plant-derived compounds that are particularly abundant in soy-based foods. Exposure to exogenous oestrogenic chemicals has been implicated in declining male fertility. The aim of this study is to deduce whether adult phytoestrogen exposure affects the reproductive function of male rats, and by what mechanisms phytoestrogens may be acting.

Six male rats were transferred from a low soy diet (control) to an experimental high soy diet, while 9 males remained on the control diet. On days 3, 6 and 12 all males were mated and litter sizes recorded. A second group of male rats kept on the same dietary regimen were killed after 3, 6 or 12 days on the diets. The epididymides were collected from the rats. Real-time PCR was performed to measure mRNA quantities of oestrogen receptors alpha (ERα) and beta (ERβ), and androgen receptor (AR). The TBARS assay for lipid peroxidation was performed on epididymal sperm samples from rats fed the high or low phytoestrogen diet for 3 days.

The average litter size following 3 days on the high soy diet was significantly lower than that for rats maintained on the control diet. Litter sizes returned to control levels by day 12. ERα and AR expression decreased in the cauda region of the epididymis following 3 days on the high soy diet, but returned to control levels by day 6. Lipid peroxidation of epididymal sperm was increased in rats fed the high phytoestrogen diet for 3 days.

Short-term exposure to high phytoestrogen levels transiently reduces male fertility, and alters hormone receptor expression. Endocrine disruption may impair fertility by reducing antioxidant protection of sperm stored in the epididymis.

GONADOTROPHIC HORMONES AFFECT THE UTERUS, IMPLANTATION AND FETAL DEVELOPMENT IN MICE


Research Centre for Reproductive Health, University of Adelaide, Woodville, SA, Australia

Although gonadotrophin stimulation using equine chorionic gonadotrophin (eCG) adversely influences pregnancy and fetal development, the effects of stimulation using recombinant human follicle stimulating hormone (rhFSH) are largely unknown. Evidence from human studies indicates that rhFSH may also be detrimental to the endometrium and implantation. We investigated the effect of gonadotrophin stimulation on ovarian hormones and uterine characteristics in the peri-implantation period, and pregnancy outcomes in mice. Adult female mice were stimulated with 2.5IU or 10IU rhFSH or 5IU eCG, followed by 5IU human chorionic gonadotrophin (hCG). Control mice received saline injections. On day 4 of pseudopregnancy mice either had embryos transferred to the uterus or were sacrificed and blood and uterine samples collected. Plasma progesterone and estradiol concentrations were determined by radioimmunoassay. Uterine mRNA levels of the estrogen and progesterone receptors (ERα and PR), leukaemia inhibitory factor (LIF), homeobox gene Hoxa10 and vascular endothelial growth factor (VEGF) were determined by real-time RT-PCR. Uterine protein distribution of PR was determined by immunohistochemistry. Embryo transfer recipients were sacrificed on day 15 to assess pregnancy outcomes. Gonadotrophin stimulation increased plasma progesterone concentration compared to controls, while estradiol concentrations were not affected. Stimulation also reduced total LIF mRNA and altered the spatial distribution of PR protein in the uterus on day 4. Embryo transfer recipients administered eCG or 10IU rhFSH had lower pregnancy rates compared to controls (11, 35 and 75% respectively) and fetuses from the rhFSH group had reduced mean weight (94 ± 6 vs 176 ± 8 mg), length (11 ± 0.2 vs 12 ± 0.1 mm) and maturity (14.1 ± 0.09 vs 14.6 ± 0.05 days) compared to controls. These results demonstrate that gonadotrophin stimulation induces changes to the maternal reproductive tract prior to implantation that have consequences for the establishment of pregnancy and fetal development in the mouse.
SUPPRESSOR OF CYTOKINE SIGNALLING 3 REGULATES IL-11 INDUCED HUMAN ENDOMETRIAL STROMAL CELL DECIDUALIZATION.
E. Dimitriadis, C. Stoikos, L. A. Salamonsen
Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

Decidualization of endometrial stromal cells is critical for embryo implantation and establishment of pregnancy. Locally produced cytokines such as interleukin (IL)-11 enhance decidualization of human endometrial stromal cells (HESC). IL-11 signaling is negatively regulated by suppressor of cytokine signaling (SOCS) proteins. IL-11 stimulates SOCS3 in human pituitary cells. The aim of this study was to examine the role of SOCS3 on IL-11 induced HESC decidualization. Decidualization of HESC was assessed using an in vitro model in which estrogen (E)+progestrone (P) or cAMP was administered for 8 days to cells. Medium was collected for prolactin (PRL) assay (a decidual marker). Cellular protein was extracted for Western analysis and cellular RNA for real-time RT-PCR analysis. SOCS3 was overexpressed in HESC cells and the effect on decidualization assessed. HESC treated with E+P or cAMP secreted PRL from day 6. Treatment of HESC with E+P or cAMP increased the abundance of SOCS3 protein, coinciding with an increase in PRL secretion. cAMP maximally stimulated SOCS3 protein and mRNA during decidualization. Antiprogestin (onapristone) added to E+P or cAMP treated cells at day 6 reduced PRL secretion but had no influence on SOCS3 abundance suggesting that SOCS3 protein was not regulated via the P-receptor pathway. Addition of IL-11 to HESC increased SOCS3 abundance from 1 hour. SOCS3 abundance returned to control levels following treatment of cells with IL-11 and IL-11 neutralising antibody. SOCS3 overexpression in HESC treated with cAMP reduced PRL secretion compared to mock- or non-transfected HESC. Furthermore, IL-11 mediated decidualization was diminished by SOCS3 overexpression. We have demonstrated for the first time that SOCS3 regulates IL-11 induced decidualization and that SOCS3 overexpression in HESC disrupts decidualization. This knowledge is important in understanding the mechanisms by which IL-11 promotes decidualization of HESC and thus the formation of decidua, an essential component of a functional placenta.

INTERLEUKIN-10 INHIBITS TNFA SYNTHESIS AND PROTECTS AGAINST LPS-INDUCED MISCARRIAGE AND PRETERM LABOUR
S. A. Robertson, R. J. Skinner, A. S. Care
Research Centre for Reproductive Health, University of Adelaide, Adelaide, SA, Australia

The immune-deviating and anti-inflammatory cytokine interleukin-10 (IL-10) is expressed throughout pregnancy in the decidual and placental tissues. Mice with a null mutation in the IL-10 gene mice are fertile with no reduction in litter size, although fetal growth trajectories and placental structure are altered. IL-10 is known to terminate inflammatory responses and to limit inflammation-induced tissue pathology by inhibiting macrophage synthesis of tumor necrosis factor-alpha (TNFα). To investigate the anti-inflammatory role of IL-10 in pregnancy, the susceptibility of null mutant mice to low dose LPS-induced miscarriage and preterm labour has been evaluated. When IL-10 null mutant C57Bl/6 (IL-10−/−) and control (IL-10+/+) mice were given low dose E.coli LPS on d10 of pregnancy, IL-10 deficiency was associated with greater fetal loss with fewer mated IL-10−/− mice carrying viable fetuses at day 18 and increased rate of fetal resorption. In mice treated with LPS on day 17, preterm delivery within 24 h occurred in a higher proportion of IL-10−/− mice than IL-10+/+ mice. LPS induced very high and sustained TNFα and IL-6 content in serum, uterine and placental tissue in IL-10−/− mice, associated with upregulated mRNA expression of both cytokines in gestational tissues. These data show that IL-10 modulates placental resistance to inflammatory stimuli by down-regulating expression of the pro-inflammatory cytokines TNFα and IL-6. We conclude that IL-10 has a dual role in pregnancy, acting to regulate placental morphogenesis and fetal growth trajectory, and to protect against inflammation-induced miscarriage and preterm labour.
EXPERIMENTALLY INDUCED HYPOGLYCEMIA: A MODEL TO EXAMINE THE EFFECTS OF LACTATION ON REPRODUCTIVE FUNCTION IN DAIRY COWS?

S. Meier¹, P. J.S. Gore¹, C. M.E. Barnett², R. T. Cursons², D. E. Phillips¹, K. A. Watkins¹, G. A. Verkerk¹

¹Dexcel Limited, Hamilton, New Zealand
²Biological Sciences, University of Waikato, Hamilton, New Zealand

The metabolic changes subsequent to lactogenesis have been associated with poor reproduction in high-producing dairy cows [1,2]. Periods of hypoglycaemia reflect severe energy deficit and are associated with changes in plasma levels of growth hormone (GH), insulin-like growth hormone-I (IGF-I) and insulin-like growth hormone-II (IGF-II). Somatotropic activity has been shown to influence reproductive functions [3-5].

This study evaluated the effects of experimentally induced hypoglycaemia in seven non-lactating cows, over a 7-day period. Phloridzin treatment (8 g/d) resulted in urinary glucose loss (control: 3.5 ± 1.0 g/d and phloridzin: 468 ± 46 g/d) and decline in plasma glucose (control: 60.6 ± 0.6 mg/dL and phloridzin: 71.8 ± 0.4 mg/dL; P<0.001). Treatment increased plasma beta hydroxybutyrate (BOH), non-esterified fatty acids (NEFA) and IGF-I concentrations (P<0.001). Plasma insulin and GH concentrations did not differ. During treatment, expression of mRNA for total growth hormone receptor (GHR(tot); P=0.012) and GHR(1A) (P<0.001) in liver tissue declined. Luteal and follicle diameters in ovaries recovered after treatment did not differ. Expressions of mRNA for IGF-I (P=0.052) and interleukin-1β (IL-1β) in corpus luteum and for 3β-hydroxysteroid dehydrogenase (3β-HSD) within dominant follicles were significantly elevated, while mRNA for GHR(tot), cytochrome P450 cholesterol side chain cleavage enzyme (P450-SCC), and steroidogenic acute regulatory protein (StAR) tended to increase (P<0.1) with treatment.

The treatment resulted in changes similar to those of nutritional stress or the initiation of lactogenesis. Phloridzin-induced hypoglycaemia may be a model to investigate mechanisms linking glucose metabolism, and the somatotropic axis to reproductive function. The advantages of such a model, is that it allows for strict control of the level of hypoglycaemia. The use of non-lactating cows also removes the feedback mechanisms that modulate mammary gland requirements, and therefore will minimize the between cow variance when using lactating cows.

This work was completed with the help from Dexcel Farms and the Dairy Cattle Fertility team. This study was funded by the New Zealand Foundation for Research, Science and Technology (DRCX 0202).

9-HODE-INDUCED APOPTOSIS IN U937 MONOCYTES IS NOT INHIBITED BY BLOCKADE OF PPARγ, AND IS ENHANCED BY ACTIVATION OF PPAR δ.
L. M. Brownrigg, K. D. Croft, I. B. Puddey, B. B. Yeap
Fremantle and Royal Perth Hospitals, School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia

The oxidation product of linoleic acid, (±)-9-hydroxy-10E,12Z-octadecadienoic acid (9-HODE) is found abundantly in oxidised LDL and in atherosclerotic lesions. 9-HODE has previously been shown by this laboratory to be a mild peroxisome proliferator activated receptor gamma (PPARγ) agonist, and to selectively induce apoptosis in U937 monocytes. Furthermore a related compound, 13-HODE, has been shown to induce apoptosis in colorectal cancer cells by down-regulating PPAR δ. The mechanism by which 9-HODE induces apoptosis in U937 monocytes is currently unknown.

Aims: To determine whether 9-HODE-induced apoptosis in U937 monocytes occurs via a PPAR γ- or PPAR δ-dependent mechanism.

Methods: U937 monocytes were treated for 48h with either 9-HODE (10μM), PPARγ antagonist GW9662 (10μM) or PPAR δ antagonist GW501516 (10μM), and combinations of either 10μM GW9662 or 10μM GW501516 with 10μM 9-HODE. Monocytes were gently harvested and briefly incubated with a mixture of Annexin-V-FITC and propidium iodide, then analysed by flow cytometry.

Results: Compared to vehicle treated cells (apoptotic RR=1), GW9662 (RR=1.46 ± 0.24, p=0.083 v vehicle (mean ± SD)) or GW501516 (RR=1.29 ± 0.01, p=0.0004 v vehicle) had modest effects on apoptosis. 9-HODE alone caused a 3-fold induction of apoptosis compared to vehicle treated cells (RR=3.16 ± 0.47, p=0.015 v vehicle), but this increased when 9-HODE was combined with either GW9662 (RR=4.16 ± 0.19, p=0.086 v 9-HODE alone) or GW501516 (RR=4.82 ± 0.48, p=0.016 v 9-HODE alone).

Conclusions: Both GW9662 and GW501516 alone resulted in small increases in apoptosis. The presence of PPAR γ antagonist GW9662 does not prevent 9-HODE related apoptosis. The PPAR δ antagonist GW501516 increased apoptosis in conjunction with 9-HODE. While 9-HODE is a PPAR γ agonist, its apoptotic action is mediated independently of PPAR γ, and is enhanced by the PPAR δ agonist GW501516.


HEATED PALM OIL IS NOT DETRIMENTAL TO BONE METABOLISM IN ESTROGEN DEFICIENT RATS
S. Ina Nirwana1, L. J. Yee2, S. F. Yew2, M. Norazlina1, S. Ahmad Nazrun1, M. Norhayati3, U. Nor Aini3, J. Kamsiah1
1Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
2Pharmacy, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
3Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia
4Parasitology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

Objectives: The effects of heated palm oil and soya oil on bone metabolism in ovariectomised Sprague-Dawley rats were studied. Methods: Sixty-four 3-month old female rats, weighing between 180-250 g were equally divided into 8 groups: normal control (NC), ovariectomised control (OVX C), fresh palm oil (POF), fresh soybean oil (SOF), once-heated palm oil (PO1), once-heated soya oil (SO1), 5x-heated palm oil (PO5) and 5x-heated soya oil (SO5). All the groups were ovariectomised except for the NC group. Rat chow fortified with the respective oils (15% w/w) was given to the tested groups. Blood and urine samples were obtained before and after four weeks of treatment. Parameters measured were serum levels of the bone resorbing cytokines, interleukin-1 and interleukin-6; as well as the bone biomarkers, serum osteocalcin (bone formation) and urine deoxypyridinolines (bone resorption). Results: Serum levels of IL-1α for all groups were undetectable. Rats in the POF and SO5 showed higher levels of IL-6 after treatment compared to the NC and OVX C (p<0.01). IL-6 was lower in the SOF group compared to the POF group (p<0.01), but higher in the SO5 group compared to the PO5 group (p<0.01). Osteocalcin levels in the NC, SOF and SO5 were decreased after treatment. The POF and PO1 group of rats had higher osteocalcin levels compared to NC. Only the POF group had lower urinary Dpd/creatinine levels after treatment. Bone remodeling index (BRI) for POF and PO1 after treatment was increased. Percentage change for BRI was higher in the POF and PO5 groups compared to the NC and SO5 groups. Conclusion: Heated palm oil has no detrimental effects on bone metabolism in estrogen deficient ovariectomised rats. On the other hand, heated soya oil induced negative changes in serum IL-6 and osteocalcin levels which may lead to osteoporosis in the long term.
AORTIC HISTOMORPHOMETRIC FINDING IN OVARIECTOMIZED RATS FED WITH HEATED VEGETABLE OILS


1Pharmacology, University Kebangsaan Malaysia, Kuala Lumpur, Malaysia
2Pathology, University Kebangsaan Malaysia, Kuala Lumpur, Malaysia
3Anatomy, University Kebangsaan Malaysia, Kuala Lumpur, Malaysia

This study examined the effects of heated vegetable oils in estrogen deficiency rats. Eighty female Sprague–Dawley rats were divided equally into eight groups (all the rats were ovariectomized except for rats in group I), each treated with the following prescribed course of food: (I) basal diet as the control (without oil) non ovariectomized, or (II) basal diet as the control (without oil), or (III) basal diet fortified with 15% weight/weight (w/w) fresh Soya bean oil (FSO), or (IV) heated once Soya bean oil (1H-
SO), or (V) heated five times Soya bean oil (5H-SO), or (VI) fresh Palm oil (FPO), or (VII) heated once Palm oil (1H-
PO), or (VIII) heated five times Palm oil (5H-PO) for 24 weeks. At the end of the study, aortic tissue was taken from consistent segment of the ascending aorta for histopathological examination. The specimens were stained with haematoxylin eosin and Verhoeff van Gieson for light microscopy. Intimal thickness was calculated using computerised image analyser. Both fresh and heated Soya or Palm oil diet did not alter the tunica intima over tunica media ratio. There was no obvious focal or diffuse atherosclerotic plaque formation seen in all groups. The intact and continuous internal elastic lamina did not support the smooth muscle cells migration as seen in atherosclerosis. There is no obvious thickening or swelling of tunica media indicates that no lipid-laden foam cells formation.

In conclusion, heated Soya and Palm oil appeared comparable in their effect on aorta. Heating did not render it more atherogenic in ovariectomized female rats. Electron microscopic study is required to see ultra structural changes.

Withdrew.

EFFECT OF HEATED SOYA AND PALM OIL ON SERUM HOMOCYSTEINE, INTERLEUKIN AND MDA IN OVARIECTOMIZED RATS

M. Norhayati, J. Kamsiah, Y. E. Lai, M. Norazliina, N. A. Umar, G. Norzana, S. Ima

1Parasitology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah Persekutuan, Malaysia
2Pharmacology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah Persekutuan, Malaysia
3Pathology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah Persekutuan, Malaysia
4Anatomy, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah Persekutuan, Malaysia

Objective: The aim of this study is to determine the changes in serum MDA, homocysteine and interleukin 6 levels of estrogen deficient rats fed with heated vegetable oils (soya and palm oil diet).

Methods: Eighty female Sprague–Dawley rats were divided equally into eight groups (all the rats were ovariectomized except for rats in group I), each treated with the following prescribed course of food: (I) basal diet as the control (without oil) non ovariectomized, or (II) basal diet as the control (without oil), or (III) basal diet fortified with 15% weight/weight (w/w) fresh Soya bean oil (FSO), or (IV) heated once Soya bean oil (1H-
SO), or (V) heated five times Soya bean oil (5H-SO), or (VI) fresh Palm oil (FPO), or (VII) heated once Palm oil (1H-
PO), or (VIII) heated five times Palm oil (5H-PO) for 24 weeks. Serum samples for interleukin and MDA were taken prior to ovariectomy and later repeated every 4 weeks post ovariectomy for 24 weeks. However, homocysteine assay was carried out prior to ovariectomy and once 24 weeks post ovariectomy.

Results: Fresh and heated Palm oil did not increase serum MDA, Homocysteine and Interleukin 6.

Conclusion: Consumption of fresh and heated palm oil did not render it more atherogenic in ovariectomized rat.
CHANGES IN SERUM LIPID PROFILES IN ESTROGEN DEFICIENT RATS FED WITH SOYA AND PALM OIL DIET

N. A. Umar1, J. Kamsiah2, J. Y.E. Lai2, M. Norazlina3, M. Norhayati3, G. Norzana4, S. Ima5

1Pathology, Universiti Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia
2Pharmacology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah, Malaysia
3Parasitology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah, Malaysia
4Anatomy, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah, Malaysia

Objective: The aim of this study is to determine the changes in lipid profiles of estrogen deficient rats fed with heated vegetable oils (soya and palm oil diet). Methods: Eighty female Sprague–Dawley rats were divided equally into eight groups (all the rats were ovariectomized except for rats in the first group; each fed with basal diet as the control (without oil), the second group fed with basal diet as the control (without oil), third group fed with basal diet fortified with 15% weight/weight (w/w) fresh Soya bean oil, fourth group with heated once Soya bean oil, fifth group with heated five times Soya bean oil, sixth group with fresh Palm oil, seventh group heated once Palm oil, and the last group with heated five times Palm oil for 24 weeks. Serum sample for lipid profile was taken prior to ovariectomy and every four weeks post ovariectomy for 24 weeks. Results: Ovariectomy increased serum level of triglyceride and total cholesterol. There is a tendency for fresh and heated Palm oil to cause transient increase in serum triglyceride, LDL-cholesterol and HDL-cholesterol. Conclusion: Consumption of fresh and heated palm oil did not render it more atherogenic in ovariectomized rat.

EFFECTS OF ANDROGENS ON MYOBLAST PROLIFERATION

Y. Chen, A. M. Axell, J. D. Zajac, H. E. MacLean

Department of Medicine, University of Melbourne, Austin Health, Heidelberg, VIC, Australia

Androgens increase the size and strength of muscle in humans. Satellite cells (quiescent myoblasts) are the major source for muscle growth and regeneration. The androgen receptor (AR) has been found in satellite cells. However, the mechanism by which androgens regulate satellite cell function remains unclear. The present study is to investigate the effects of androgens on the proliferation of myoblasts in vitro, using C2C12 and Sol8 cells, two well established myoblast cell lines from mouse skeletal muscle. Firstly, AR expression was identified in these two cell lines by reverse transcript PCR (RT-PCR). Then the conditions for proliferation of cells were optimised. Cells were cultured in charcoal strip fetal calf serum (CS-FCS) with the addition of androgen every 24 hours. The MTT assay was used to quantitate proliferation of cells. No significant effect of androgens on proliferation of C2C12 and Sol8 was observed using either testosterone or dihydrotestosterone, at concentrations ranging from 10-6 to 10-9 M for up to 6 days of treatment. However, C2C12 cells treated with 10 ng/ml IGF-I showed a higher proliferation rate compared with controls, indicating the cells are capable of responding to a mitogenic stimulus. Levels of AR protein in the cell lines were investigated. No AR protein was detectable in either cell line by Western analysis, indicating that protein levels of the AR were below the limit of detection. This low level of AR protein might explain the lack of response of the myoblast cell lines to androgen treatment. In order to further study androgen actions in myoblasts in vitro, we have stably transfected 13 C2C12 cell lines to overexpress the mouse AR. We have confirmed AR protein levels are detectable in 7 cell lines, and are currently examining the effects of androgens on proliferation and differentiation in myoblasts in these cell lines.
GONADOTROPHIN-INHIBITORY HORMONE (GNIH) SUPPRESSES LH SECRETION IN THE RAT
G. M. Anderson, F. J. Steyn
Centre for Neuroendocrinology and Dept of Anatomy and Structural Biology, University of Otago, Dunedin, New Zealand

The secretion of the gonadotrophins, LH and FSH, and hence the control of fertility is driven by gonadotrophin-releasing hormone (GnRH). The concept of direct inhibition of pituitary gonadotrophin secretion by the brain was unknown until very recently, when a ‘new’ hypothalamic hormone, which suppressed gonadotrophin release from cultured quail pituitary glands, was discovered and named GNIH. Since we have observed GnIH-immunoreactive cells in the rodent brain as well as fibres projecting to the median eminence, we hypothesized that GnIH acts at the pituitary gland to suppress gonadotrophin secretion in mammals as well as birds. In Experiment 1, sparrow GnIH-related peptide 2 (GnIH-RP2; 10 µg) or vehicle was injected iv into ovariectomized rats. Blood was collected at 0, 1.5, 3 and 10 minutes for LH analysis. After 3 minutes, plasma LH was suppressed to 50% of basal values by GnIH-RP2 (P < 0.05). To test if GnIH inhibited GnRH-induced LH secretion (Experiment 2), GnRH (0.1 µg) was injected iv either alone or with GnIH-RP2. Rats were blood sampled as before. GnRH caused a robust release of LH, but in the presence of GnIH the peak LH response was suppressed to 60% of control values (3 minutes after injection; P < 0.05). We also tested whether GnIH can affect pulsatile GnRH release (Experiment 3), since we and others have observed GnIH fibres projecting to GnRH cell bodies. Rats were blood sampled every 10 minutes for 4 hours. GnIH (1 µg) or vehicle was injected icv after 2 hours. LH pulse frequency (an index of GnRH pulse frequency) was unaffected by GnIH. These results support a neuroendocrine role for GnIH and are the first to suggest that a direct inhibitory system governing pituitary gonadotrophin release exists in mammals. However, they do not support a role for GnIH in central regulation of GnRH.

OREXIN RECEPTOR SUBTYPES-1 AND -2 EXHIBIT DISTINCT BETA-ARRESTIN PROFILES DETERMINED USING BIOLUMINESCENCE RESONANCE ENERGY TRANSFER (BRET) AND CONFOCAL MICROSCOPY
M. B. Dalrymple1, K. D.G. Pfleger1, E. M.L. Lim1, K. A. Eidne1
1Laboratory for Molecular Endocrinology, WA Institute for Medical Research and Centre for Medical Research, University of, Nedlands, WA, Australia
2School of Anatomy and Human Biology, University of Western Australia, Nedlands, WA, Australia
3Keogh Institute for Medical Research, QEI 1 Medical Centre, Nedlands, WA, Australia

Since their discovery in 1998, the role of orexin neuropeptides has been the subject of considerable interest. While familial canine narcolepsy is clearly correlated with functionally null type-2 orexin receptor (OxR2), the human condition is less clearly defined. By examining the two orexin receptor subtypes and their interactions with beta-arrestins 1 (Barr1) and 2 (Barr2), we aim to gain a greater understanding of the desensitization/internalization mechanisms regulating this system. A comprehensive series of BRET dose-response assays were performed, using both OxR1 and OxR2 in combination with Barr1, Barr2 and the respective phosphorylation-independent mutants. These data show no significant differences between Barr1 and Barr2 for these receptors, in keeping with their classification as Class B for beta-arrestin usage. Orexin B (OxB) is selective for OxR2 over OxR1 and our data showing much greater affinity of beta-arrestins for OxB-activated OxR2 reflects this selectivity. Interestingly, despite Orexin A (OxA) demonstrating similar efficacy for both OxR1 and OxR2, our data shows a significantly greater affinity of beta-arrestins for OxA-activated OxR2 compared with OxR1. Furthermore, phosphorylation-independent beta-arrestins had a significantly greater affinity for OxR1 than wild-type beta-arrestins, whereas this was not the case for OxR2. This suggests that OxR2 possesses additional components compared to OxR1 that enable stronger interactions with beta-arrestins and that phosphorylation-independence at least partially overcomes the need for these additional components. This receptor-specific difference correlates with our extended BRET kinetic studies showing a more robust beta-arrestin interaction with OxA-activated OxR2 compared with OxR1 over time. Confocal microscopy studies correlate with the BRET data, with receptor and/or beta-arrestin translocation evident for both OxA-activated receptors and OxB-activated OxR2, but little if any translocation evident with OxB-activated OxR1. Our results indicate receptor subtype-specific differences in beta-arrestin regulation of orexin receptor desensitization/internalization, providing a mechanism for distinct roles in the intricate functioning of the orexigenic system.
PRENATAL BETAMETHASONE EXPOSURE SIGNIFICANTLY ALTERS FETAL ADRENAL STEROIDOGENIC ENZYME P450C17 GENE EXPRESSION IN SHEEP

S. Li, J. P. Newnham, J. R. G. Challis, T. J. M. Moss, D. M. Sloboda
1School Of Women's and Infants' Health, The University of Western Australia, Subiaco, WA, Australia
2King Edward Memorial Hospital, Women and Infants Research Foundation, Subiaco, WA, Australia
3Physiology and Obstetrics and Gynecology, The University of Toronto, Toronto, Ontario, Canada

Fetal exposure to synthetic glucocorticoids increases fetal and postnatal hypothalamic-pituitary-adrenal (HPA) axis activity. Peri-conceptional undernutrition alters fetal adrenal receptor and steroidogenic enzyme expression, but little is known about the effects of fetal exposure to synthetic glucocorticoids on adrenal cortisol synthesis. Our aim was to determine the levels of fetal adrenal steroidogenic enzyme P450c17 mRNA after one, two or three doses of betamethasone administered to the mother. Pregnant ewes were injected with saline or 1 (104 days of gestation; dG), 2 (104, 111 dG) or 3 (104, 111, 118 dG) doses of betamethasone. Fetal blood samples and fetal and postnatal pituitary and adrenal tissue was collected at 84 (control only), 109, 116, 133 dG and 6 weeks postpartum. Plasma was collected for ACTH and cortisol (F) analyses. Fetal pituitaries and adrenals were collected and adrenals processed for mRNA determination of steroidogenic enzyme P450c17 (real time RT-PCR). One dose of maternal betamethasone reduced fetal pituitary weight at 109 dG (P=0.027) and three doses decreased pituitary and adrenal weights at 133 dG (P=0.052; P=0.01). Three doses of betamethasone resulted in a modest increase in adrenal weight at 6 weeks of age (P=0.08). Plasma ACTH and F levels increased with increasing pre- and postnatal age in both groups (P=0.001). ACTH levels were reduced at 6 weeks of age after 3 doses of betamethasone (P=0.038). Adrenal P450c17 mRNA levels increased with increasing pre- and postnatal age in both groups (P=0.001). P450c17 mRNA levels were reduced at 109 dG after one dose of betamethasone (P=0.004), and modestly reduced after two (at 116 dG; P=0.1) or three doses (at 133 dG; P=0.057). The ratio of F to P450c17 mRNA increased with increasing pre- and postnatal age in both groups but was higher after one dose (at 109 dG; P=0.004), two doses (at 116 dG; P=0.03) or three doses (at 133 dG; P=0.029) of betamethasone. Fetal exposure to betamethasone alters fetal adrenal P450c17 enzyme expression. An increase in F: P450c17 may be indicative of an increase in adrenal efficiency in producing cortisol or alternatively a reduction in cortisol clearance.


USE OF A DIURNAL CORTISOL BLOOD SPOT PROFILE FOR ADJUSTMENT OF HYDROCORTISONE DOSE.

H. D. Russell, S. M. Lau, M. McLean

Center for Diabetes and Endocrinology Research, Dept of Diabetes and Endocrinology, Westmead Hospital, NSW

Adjustment of glucocorticoid replacement therapy for patients with pituitary or adrenal disease based on clinical evaluation can be imprecise. Obtaining a diurnal profile of cortisol levels requires multiple venepunctures. We have developed a method for obtaining a diurnal profile of cortisol concentrations from dried capillary bloodspots collected at home by the patient. This study evaluates the use of this method to adjust hydrocortisone replacement regimens. We further assess the effect of dose adjustment on bone turnover and peripheral white blood cell counts; markers of end-organ response to glucocorticoid action. This method utilises methanol extraction and reconstitution in an assay buffer to allow measurement of cortisol from small volume samples in a standard commercial assay (DPC Immulite 2000). Results from the bloodspot assay were validated against simultaneously collected serum cortisol measurements collected by venepuncture, yielding a highly significant linear correlation (r=0.93, n=80) across a range of serum cortisol concentrations of 83 – 659 nmol/l. The relationship y=0.138x was subsequently used to transform bloodspot cortisol concentrations to equivalent serum cortisol estimations. In a single day, 10 patients with either primary or secondary hypoadrenalism who were clinically glucocorticoid sufficient, collected 5-6 capillary blood samples by fingerplick, absorbed onto blotting paper. The samples were obtained before and 2 hours after each hydrocortisone dose. The dried blotting paper was mailed to our laboratory. Patients were taking either twice or thrice daily hydrocortisone replacement (total daily dose range 12mg-30mg). Dose adjustment was made according to predetermined target ranges based on expected physiological serum cortisol concentrations. Doses adjustments were required in 7 patients. 2 patients were changed to thrice daily dosing. Data will be presented on changes in urinary deoxyepiridolione and white cell counts following hydrocortisone dose adjustment. Bloodspot cortisol measurement enables convenient and effective optimisation of hydrocortisone replacement therapy, detecting subclinical under or over-replacement.
EFFECTS OF TOCOTRIENOL AND TOCOPHEROL ON CORTICOSTEROONE LEVEL AND GASTROINTESTINAL CHANGES IN RATS EXPOSED TO STRESS

M. Nur Azlina, K. Rubaizah, M. Muliana, M. Nafeeza
Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Wilayah Persekutuan, Malaysia

This study investigates the effects of supplementation with either tocotrienol (TT) or tocopherol (TF) on corticosterone level and gastrointestinal changes in rats exposed to restraint stress. Twenty-four male Sprague-Dawley rats were randomly assigned into 4 equal sized groups, two control groups were given a commercially prepared normal rat diet, while the treated group was fed with an identical diet with supplementation of either tocotrienol of tocopherol orally at the dose of 60mg/kg body weight. After 28 days of treatment, one control group, TT group and TF group were subjected to restraint stress, 2 hours daily for 4 consecutive days. After the last exposure to stress, plasma samples were taken to determine the corticosterone level and after which the rats were killed. The stomach was excised for the evaluation of gastric lesion, malondialdehyde (MDA) and prostaglandin E<sub>2</sub> levels. The results showed that TT and TF were able to maintain the corticosterone level to the pre-stress values in rats exposed stress. Tocotrienol was found to be better in preventing formation of gastric lesion compared to rats supplemented with TF while both TT and TF were proved to significantly reduce the gastric MDA content in stress condition compared to control group. We also found that both TT and TF have the ability to reduce prostaglandin E<sub>2</sub> loss which was apparent with stress exposure. As a conclusion, tocotrienol and tocopherol are capable in reducing stress-induced damages in the gastric tissue probably through inhibiting stress induced elevation of corticosterone levels as well as through free radical scavenging activities.

A COMPARISON OF BODY COMPOSITION IN CUSHING'S SYNDROME AND GROWTH HORMONE DEFICIENCY

M. G. Burt<sup>1,2</sup>, J. Gibney<sup>1</sup>, K. K.Y. Ho<sup>1,2</sup>
<sup>1</sup>Pituitary Research Unit, Garvan Institute of Medical Research, Sydney, NSW, Australia
<sup>2</sup>Department Of Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia

Glucocorticoid excess and growth hormone deficiency (GHD) both perturb body composition by increasing fat mass (FM) and reducing lean body mass (LBM). Because these occur via different metabolic mechanisms, it is likely that distinct differences in the severity and distribution of body composition abnormalities exist. The aim was to compare body composition in subjects with Cushing's syndrome (CS) and GHD. Eighteen subjects with CS (12F, age=41.5±3.0yrs, 24h UFC=1471±329nmol/d, normal=300nmol/d), 22 subjects with GHD (14F, age=42.9±2.9yrs) and 18 normal subjects (11F, age=46.8±2.8yrs) were studied. LBM, FM, bone mineral content (BMC) and regional body composition analysis were assessed by DXA. In both CS and GHD, FM was significantly greater and LBM significantly lower than normal subjects (Table). There were no significant differences in FM and LBM between subjects with CS and GHD. BMC was significantly lower in CS, but not GHD, than normal subjects. In CS, limb lean tissue was significantly less than both groups. Truncal fat was significantly greater than normal subjects in CS and tended to be greater than in subjects with GHD. In summary FM is increased and LBM reduced in both CS and GHD. However CS results in greater perturbation of regional body composition, with a greater reduction in limb lean mass and a greater increase in truncal fat. This may result in subjects with CS having greater functional impairment and a less favourable metabolic profile.

<table>
<thead>
<tr>
<th></th>
<th>Weight (kg)</th>
<th>FM (%)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>LBM (%)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>BMC (%)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Limb lean mass (%)&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Truncal fat (%)&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>72.4±4.2</td>
<td>33.8±2.4</td>
<td>62.1±2.3</td>
<td>4.1±0.1</td>
<td>29.7±1.1</td>
<td>15.8±1.2</td>
</tr>
<tr>
<td>CS</td>
<td>75.1±3.6</td>
<td>43.9±1.6</td>
<td>52.7±1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.4±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.3±1.3&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>GHD</td>
<td>76.9±2.9</td>
<td>41.1±2.1</td>
<td>55.1±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8±0.13</td>
<td>26.5±1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.2±1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*</sup> Expressed as % of body weight
a = p<0.05 vs normal subjects
b = p<0.05 vs GHD
c = p<0.01 vs GHD
ACTIVIN AS A NOVEL MARKER IN CLINICAL INFLAMMATORY PROCESSES: ELEVATIONS IN BURNS AND TRAUMATIC BRAIN INJURY PATIENTS.


1Monash Institute of Medical Research, Monash University, Clayton, VIC, Australia
2Burns Unit, Alfred Hospital, Melbourne, VIC, Australia
3Department of Neurosurgery, Alfred Hospital, Melbourne, VIC, Australia
4National Trauma Research Institute, Melbourne, VIC, Australia

Actinin A is a member of the Transforming Growth Factor-β superfamily with diverse roles, but it has only been recently identified as a cytokine elevated in inflammatory processes. In animal models of acute inflammation, actinin A is released into the circulation within 40 minutes of challenge with agents such as lipopolysaccharide. The relevance of this response to clinical inflammatory syndromes was confirmed where circulating actinin levels were high in patients with septicemia. However, whether this applies to other clinical settings of acute inflammation is not currently known. Here, the response of actinin was assessed in two major inflammatory conditions, in patients who have suffered a major burns episode and those that have undergone a traumatic brain injury (TBI), typically following a vehicle accident. In burns patients, actinin was elevated both systemically in the circulation and locally in burns blister fluid as assessed by an immunohistochemistry specific for actinin A. Tissue immunohistochemistry using an antibody specific for the actinin βA subunit (actinin A is a dimer of two βA subunits) showed that fibroblasts in the dermis of the burns wound were immunopositive for actinin, along with infiltrating leukocytes and neutrophils. In TBI, actinin A was elevated in a subset of patients in the cerebrospinal fluid (CSF), but there were only minor or no elevations in the systemic circulation. There were no apparent relationships between the degree of actinin response and coma/injury score, but CSF actinin was correlated with markers of ischaemia and/or neural injury. Furthermore, the length of stay in intensive care was associated with the degree of actinin response in the CSF. These findings add further evidence that actinin is upregulated in a number of clinical inflammatory settings and offer new perspectives in terms of potential diagnostic and/or therapeutic opportunities involving this protein.

TESTOSTERONE ADMINISTRATION PREVENTS SKELETAL MUSCLE ATROPHY AND ENHANCES RESISTANCE TO FATIGUE IN ORCHIDECTOMISED MALE MICE

A. Axell, H. E. MacLean, D. Plant, L. J. Harcourt, M. Jimenez, D. J. Handelsman, G. S. Lynch, J. D. Zajac

1Medicine, Austin Health, University of Melbourne, Heidelberg, VIC, Australia
2Physiology, University of Melbourne, Parkville, VIC, Australia
3ANZAC Research Institute, Sydney, NSW, Australia

The mechanism of androgen action in muscle growth and function remains poorly understood. Our aim is to investigate the effect of androgens on muscle mass and function using an in vivo androgen withdrawal/replacement mouse model. Eight week-old C57BL/6 male mice were orchidectomised (Orx) or sham-operated (Sham) and treated for 10-11 weeks with 3 intraperitoneal testosterone (T) or vehicle (V) implants (n=6/group). Maximum force and fatigue of fast-twitch (extensor digitorum longus; EDL) and slow-twitch (soleus; SOL) hind limb muscles were determined by in vitro muscle function testing. Histology was performed to assess fibre cross-sectional area (CSA) and fibre type distribution. Orx+V mice had decreased mass of the androgen-responsive seminal vesicles (87%, p<0.005) and levator ani (LA) muscle (95%, p<0.001) compared to Sham+V, and this was prevented with testosterone treatment. Sham+T mice had greater mass of both fast (EDL; 25%, p<0.001) and slow-twitch (SOL; 30%, p=0.001) muscles compared to Orx+V mice, and this was accompanied by greater maximum force production (EDL; 25%; SOL, 25%) (p<0.05). Orx+T mice had a 2-fold increase in fatigue resistance of slow-twitch (p<0.05) but not fast-twitch muscles compared to Orx+V controls. In the LA, orchidectomy-induced muscle atrophy correlated with a decrease in fibre CSA (p<0.001), and this was prevented with testosterone treatment. Proportion of fast-twitch fibres in the SOL muscle was significantly decreased in Orx+V mice compared to Sham+V controls (p<0.05). Assays for metabolic enzyme activity are underway to further investigate the role of androgens in regulation of muscle fibre type characteristics. We have demonstrated that muscle mass and force in male mice is androgen-dependent, and that orchidectomy-induced muscle atrophy is prevented with testosterone treatment. Our results indicate that androgen-induced changes in muscle mass lead to a proportional change in muscle force. In slow-twitch muscle, androgens alter fatigue resistance and fibre type proportion.
EFFECTS OF LOW DOSE ESTRADIOL SILASTIC IMPLANTS IN AROMATASE DEFICIENT (ARKO) MICE.

M. Jimenez, M. Jones, S. McPherson, G. Risbrider, J. Spaliviero, C. Allan, D. Handelsman

1Andrology laboratory, ANZAC Research Institute, Concord Hospital and University of Sydney, NSW, Australia
2Prince Henry Institute of Medical Research, Clayton, VIC, Australia
3Monash Institute of Medical Research, Clayton, VIC, Australia

The vital role of estrogen in male as well as female physiology, notably in gonads, bone, brain and fat, has been studied using estrogen deficient mice created by global knock-out of the unique aromatase enzyme which mediates irreversible conversion of testosterone (T) to estradiol (E2). Studies of physiological E2 replacement in males are limited by the ability to deliver very low E2 doses. We developed silastic implants that deliver low dose E2 to maintain physiological blood E2 in castrate mice for prolonged periods(1). To verify E2 effects in male mice lacking non-gonadal sources of E2 production, we examined these E2 implants in global aromatase knock out (ArKO) mice.

Male ArKO mice at 8-10 weeks had subdermal insertion of a 1cm silastic implant filled with E2 recrystallized and diluted with cholesteral for 2 or 4 weeks, a dose maintaining physiological blood E2 levels for >6 weeks in castrate normal male mice (1). Untreated wild type (WT) and ArKO male mice were controls. Increased serum T in ArKO mice (49±9 nM) was normalized at 2 (12±3nM) and 4 (14±3 nM) weeks compared with WT (16±4 nM). Similarly, E2 treatment reduced the increased seminal vesicles in ArKO towards that of WT mice while testis weight was unaffected by E2 deficiency or treatment. Prostate lobes were hypertrophied in control ArKO mice but displayed lobe-specific response to low dose E2 treatment, with ventral, anterior and dorsal lobe weights suppressed by E2 treatment towards WT levels. By contrast, the lateral prostate lobe was unaffected by aromatase deficiency or E2 treatment. This study using the congenitally estrogen deficient ArKO male mice demonstrates prostate lobe-specific responses to E2 deficiency and treatment while further validating the modified silastic implants for the sustained delivery of low dose, physiological E2 levels.

(1) Spaliviero et al Proceedings ESA 2004, Sydney

INHINIB-ALPHA IN PROSTATE CANCER: TUMOR SUPPRESSOR AND PRO-METASTATIC FACTOR


1Centre for Urological Research, Monash Institute of Medical Research, Melbourne, VIC, Australia
2Bernard O'Brien Institute of Microsurgery, St Vincent's Hospital, Melbourne, VIC, Australia
3Department of Oncology, University of New South Wales, Sydney, NSW, Australia
4Department of Endocrinology, Leiden University of Medical Centre, Leiden, Netherlands

Inhibin-alpha (INHA) has been shown to act as a tumor suppressor in mice yet is elevated in women with ovarian cancer. Similarly, studies investigating the role of INHA in prostate cancer (PCa) yield conflicting results. We have previously reported the down-regulation of INHA in PCa yet prostate tumors from patients with high risk of recurrence have been reported to show increased INHA expression.

We have suggested a unifying hypothesis that addresses this apparent contradiction and propose that INHA may function as a tumor suppressor in the early stages of tumorigenesis but switches to become a pro-metastatic factor during later stage disease. This study examines the effect of expressing INHA in two INHA negative prostate cancer cell lines, which represent androgen dependent, well differentiated, poorly tumorigenic LNCaP cell line and androgen independent, poorly differentiated highly tumorigenic PC3 cell line.

In vitro and in vivo functional studies using LNCaP cells demonstrated over-expression of INHA significantly reduces rate of cell growth, proliferation and tumor size. This supports INHAs role as a tumor suppressor in the early stages of tumorigenesis. Similar studies involving PC3 cells over-expressing INHA demonstrated significantly increased rate of cell growth and proliferation in vitro consistent with pro-metastatic or loss of tumor suppressive role of INHA, but invasive capacity, tumor size and the incidence of bone lesions were not increased, rather they were decreased (consistent with tumor suppressive effects); orthotopic inoculation showed increased tumor size and increased incidence of lymph node metastasis. This supports the role of INHA as a pro-metastatic factor but indicates that this role maybe tissue specific. The increase in lymph node metastasis was further supported by increased in lymphangiendo genesis factor (VEGF-C) in the cells.

Overall, this study demonstrates that the biological function of INHA is dependent on the stage of carcinogenesis thereby supporting the hypothesis and indicates that there is indeed a correlation between INHA levels and end stage PCa but the precise role of INHA during this stage is dependent on the environment.
TESTICULAR HYPTERTROPHY AFTER HEMICAstration IN THE NEONATAL BOAR REQUIRES GONADOTROPHIN BUT NOT TESTOSTERONE SUPPORT

R. Wells¹, S. D. Johnston¹, T. E. Trigg², M. J. D'Occhio¹

¹School of Animal Studies, The University of Queensland, Gatton, QLD, Australia
²Peptech Animal Health Pty Ltd, North Ryde, NSW, Australia

Circulating concentrations of testosterone were unchanged after hemicastration in boars (1) implying that the synthesis and secretion of testosterone by the remaining testis was increased. To determine the requirement for gonadotrophin and testosterone support for testicular hypertrophy after hemicastration in neonatal boars animals were assigned at birth to one of six treatments (n = 5 per treatment): Group 1, control, no treatment; Group 2, implanted with a gonadotrophin releasing hormone (GnRH) agonist (Deslorelin) within 24h of birth (Day 0) to suppress gonadotrophins; Group 3, hemicastrated on Day 10; Group 4, implanted with agonist (Day 0) and hemicastrated (Day 10); Group 5, implanted with agonist (Day 0) and hemicastrated (Day 10); Group 6, implanted with agonist (Day 0), hemicastrated (Day 10) and treated with testosterone from Day 10. The GnRH agonist Deslorelin (Peptech Animal Health Pty Ltd) was administered as a controlled-release implant (10-20 m g/24h) and testosterone (VR Testoprop, Jurox Pty Ltd) was injected i.m. (100mg) on alternate days from Day 10-24. The right testis was removed on Day 25. Treatment with agonist from Day 0 (Group 2, 4) or Day 10 (Group 5) suppressed (P<0.01) testicular growth, irrespective of whether boars were also hemicastrated (Group 4, 5). Hemicastration alone (Group 3) resulted in an enhanced rate of testicular growth and on Day 25 the right testis (6.7 ± 0.8g; mean ± SEM) was heavier (P<0.01) than in controls (4.3 ± 0.4g). Boars implanted with agonist, hemicastrated, and treated with testosterone (Group 6) did not show testicular hypertrophy and on Day 25 had a smaller (P<0.01) testis (0.8 ± 0.1g) than controls. The epididymis (an androgen dependent organ) for boars in Group 6 was similar (1.4 ± 0.2g) to controls (1.2 ± 0.1g). It was concluded that testosterone is not essential for testicular hypertrophy after hemicastration in boars but gonadotrophin support is necessary.

(1) Theriogenology 1981; 16: 249-257

---

OVARIAN STATUS AND EMBRYO YIELDS IN SUPEROVULATED EWES: THE EQUILIBRIUM BETWEEN ABSENCE OF DOMINANT FOLLICLES AND PRESENCE OF SIZE-ADEQUATE FOLLICLES

A. Velga-Lopez¹, M. J. Cocero¹, V. Dominguez¹, A. S. McNeilly¹, A. Gonzalez-Bulnes¹

¹Dpto. Reproducción Animal, INIA, Madrid, Spain
²Centre for Reproductive Biology, MRC, Edinburgh, United Kingdom

In sheep, ideal ovarian conditions for higher embryo yields are related to the presence of a high number of gonadotrophin-responsive follicles at start the superovulatory treatment (1), in absence of a dominant follicle (2). However, there are no studies regarding how size of subordinate follicles may affect the embryo outputs. Evaluation of such effect was carried out in forty-three Manchega ewes treated with an intravaginal progestagen for 14 days and superovulated with eight decreasing doses of oFSH (OVAGEN™), starting on Day 12 after sponge insertion. The diameter of the largest follicle (LF1) and the second largest follicle (LF2) were determined by ultrasonography at the first two FSH doses (0 and 12 hours, respectively). Embryos were recovered and evaluated on Day 21. Neither size of LF1 nor size of LF2 were found to affect ovulation rate; however, a higher size of both largest follicles decreased recovery rates (r = 0.608, P < 0.05 for LF1 and: r = 0.884, P < 0.05 for LF2). Thereafter, embryo viability was also negatively affected by a larger LF1 due to a lower fertilization rate and a higher number of degenerated embryos (r = 0.464, P < 0.05 and r = 0.509, P < 0.05, respectively). Conversely, a lower LF2 at both first FSH doses was found to be related to a decreased embryo viability (r = 0.877, P < 0.05). Current results confirmed previous reports of effects of dominant follicles on rates of embryo recovery, fertilization and degeneration. However, is the first report that highlights the role of the size limit of accompanying follicles; the second large follicle may exert co-dominance whilst too small subordinate follicles ovulate, but may be too immature to give rise a viable embryo.

(1) Gonzalez-Bulnes et al. Theriogenology; 2003; 60:281–8
(2) Brebion and Cognie 1989, 5th AEETE Proc
EXPRESSION PROFILES OF STEROID RECEPTOR-ASSOCIATED IMMUNOPHILINS IN HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE
C. L. M. Clunining, B. K. Ward, J. M. Bentel, L. A. Salamonson, T. Ratajezak
1Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia
2Laboratory for Molecular Endocrinology, UWA Centre for Medical Research, Nedlands, WA, Australia
3Department of Anatomical Pathology, Royal Perth Hospital, Perth, WA, Australia
4Prince Henry's Institute of Medical Research, Monash University, Clayton, VIC, Australia

Hormone-free steroid receptors assemble into heterocomplexes with several molecular chaperone components, including the major heat shock protein Hsp90 and Hsp90-associated co-chaperones such as the immunophilins cyclophilin 40 (CyP40), FKBP51, FKBP52 and PP5. There is emerging evidence that these immunophilins have a role in modulating receptor responsiveness to hormone. This may be determined by the relative expression of these proteins within target cells with certain immunophilins (e.g. FKBP52) acting to potentiate receptor activity and others (e.g. FKBP51) to attenuate receptor function. In addition to their pivotal role in hormonal signalling, the steroid receptor-associated immunophilins are themselves hormonally regulated. Human endometrium undergoes profound changes through the course of the menstrual cycle, largely attributable to the effects of estrogen and progesterone. The aim of the present study was to determine the expression profiles of the immunophilin co-chaperones in human endometrium throughout the menstrual cycle.

Using immunohistochemical techniques we have preliminary data that shows expression of the immunophilins FKBP52 and CyP40 in glandular epithelium follows a parallel expression pattern, revealing elevated expression during the secretory phase of the menstrual cycle. With FKBP52, increased expression was evident from the early secretory phase and remained elevated through to the late secretory phase. CyP40 expression was more variable during the secretory phase, with maximal expression occurring in mid-secretory phase and then decreasing slightly in the late secretory phase. In the stroma there was a trend to a more gradual increase in expression levels of both FKBP52 and CyP40 from the proliferative phase through to the late secretory phase. For both immunophilins staining was more intense in the glandular epithelium relative to stromal tissue. Our data suggest that CyP40 and FKBP52 might be required to support further growth and development of secretory phase endometrium. Expression profiling of FKBP51 and PP5 is in progress.

A PHARMACOGENOMIC APPROACH TO THE TREATMENT OF INFERTILITY IN POLYCYSTIC OVARIAN SYNDROME
A. J. Umbers, N. Johnson, A. N. Shelling
Obstetrics and Gynaecology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

Polycystic ovarian syndrome (PCOS) is a very prevalent and heterogeneous disease, affecting five to ten percent of women. The etiology of PCOS remains unknown; however, genetic involvement is recognised. Patients suffer significant reproductive and metabolic morbidity. Obesity is strongly associated with the disease, and in New Zealand, obese individuals are ineligible for the standard infertility treatment (clomiphene citrate). As these patients have very few treatment options and poor fertility prognosis, there has been a significant drive to treat these patients by other means.

Recently, metformin, an insulin sensitising agent, has been used to treat the metabolic and endocrine abnormalities of PCOS. Small scale observational studies indicate that only 60% of women ovulate in response to metformin. Currently, no parameters exist to predict which patients will be sensitive to metformin therapy. The aim of this research was to investigate novel genetic and phenotypic markers of therapeutic outcome to metformin treatment.

In collaboration with the first large scale controlled trial of metformin at National Women's Hospital, Auckland, New Zealand, pharmacogenomics was investigated as an predictor of fertility response in an obese PCOS patient cohort. Genetic analysis of polymorphisms in candidate genes revealed significant predictive value of polymorphisms in two genes, namely the steroidogenic enzyme, CYP17, and insulin receptor substrate-2. Phenotypic markers of insulin resistance also appeared to predict drug response, where a higher degree of insulin resistance was associated with a positive therapeutic response to metformin.

Preliminary results of this novel study warrant further investigation. Pharmacogenomic and phenotypic predictors may identify patients sensitive to therapy prior to treatment. Clinical application of this approach is likely to have a substantial impact on the treatment of PCOS, and hence help alleviate the clinical and social morbidity that is caused by this prevalent reproductive disorder.
THE ENDOCRINE RESPONSE OF MAIDEN EWES TO THE RAM EFFECT IS NOT DEPENDENT ON PRIOR EXPERIENCE WITH RAMS

P. A.R. Hawken1,2, A. C.O. Evans3, A. P. Beard2
1Faculty of Natural and Agricultural Science, University of Western Australia, Crawley, WA, Australia
2School of Agriculture, Food and Rural Development, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom
3Department of Animal Science, University College Dublin, Dublin, Ireland

Murtagh et al., (1984) found that pre-exposure of maiden ewes to rams during anoestrus increased the proportion of ewes ovulating in response to the ram effect. This study tested whether pre-exposure of maiden male ewes to rams during mid-anoestrus would enhance their endocrine response when subsequently introduced to rams during late anoestrus. During mid-anoestrus (June) ewes were kept with rams for 7 days (RE, ram-experienced; n=6) or isolated from ram contact (RN, ram-naive; n=6). All ewes were subsequently isolated from ram contact. During late anoestrus (September), the ewes were introduced to rams midway through a frequent blood-sampling regime (every 12 minutes for 12 hours) Both RE and RN ewes had a significant increase in LH pulse frequency and basal LH concentrations in response to ram introduction. RE ewes had a significant increase in mean LH concentrations and this response tended towards significance in RN ewes. Overall there was no significant effect of prior ram experience on the LH response or on the proportion of ewes having an LH surge. In conclusion, the endocrine response to the ram effect is not dependent on and does not appear to be greatly enhanced by prior ram experience.


<table>
<thead>
<tr>
<th></th>
<th>RN</th>
<th>RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsed per 6 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before RE</td>
<td>1.20 ± 0.50</td>
<td>0.40 ± 0.24</td>
</tr>
<tr>
<td>After RE</td>
<td>3.29 ± 0.57</td>
<td>4.40 ± 0.81</td>
</tr>
<tr>
<td>Mean concentration (µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before RE</td>
<td>0.27 ± 0.05</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>After RE</td>
<td>1.07 ± 0.49</td>
<td>1.40 ± 0.20</td>
</tr>
<tr>
<td>Pulse amplitude (µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before RE</td>
<td>1.01 ± 0.69</td>
<td>1.47 ± 0.47</td>
</tr>
<tr>
<td>After RE</td>
<td>1.71 ± 0.80</td>
<td>2.18 ± 0.96</td>
</tr>
<tr>
<td>Basal concentration (µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before RE</td>
<td>0.11 ± 0.08</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>After RE</td>
<td>0.55 ± 0.23</td>
<td>0.64 ± 0.51</td>
</tr>
</tbody>
</table>

SECRETION PATTERNS OF LH AND FSH AROUND ESTRUS IN MITHUN (BOS FRONTALIS)

A. Dhall1, D. P. Mishra2, A. Mech1, M. Karunakaran3, H. Choudhury1, C. Rakhawa1
1Division of Animal Production and Management, National Research Centre on Mithun, Medipharma, Nagaland, India
2Sperm Biotechnology Laboratory, National Institute of Immunology, New Delhi, India
3Division of Animal Reproduction, ICAR-RC-NEH Region, Medipharma, Nagaland, India

Mithun (Bos frontalis) a semi-domesticated bovine, reared primarily for beef, is mainly distributed in parts of India, Myanmar, Bhutan, China and Bangladesh. At present no information is available on the secretion pattern of gonadotropins around estrus in mithun. The study was conducted on 10 adult mithuns to establish the secretion patterns of LH and FSH around estrus. From estrous onset jugular vein blood samples were collected every 2 hr for 72 and 96 hr, respectively from animals without and with standing heat to determine the plasma concentrations of LH and FSH.

Frequent short-duration low-amplitude LH and FSH surges were observed throughout the estrous period. Average LH concentration (ng/ml) on the day of estrous onset was found higher in animals with (7.1±0.9) or without (7.1±0.8) standing heat but was insignificant. The amplitude of peak LH concentration (ng/ml) and interval (h) between estrous onset to peak LH did not differ significantly in animals with (15.8±0.4, 37.0±9.9) or without (16.4±1.2, 42.3±11.6) standing heat. Average FSH concentration (ng/ml) did not vary significantly among different days of estrus in animals without standing heat. But it was found significantly (p<0.01) higher (6.8±0.4) on the second day of estrus in animals with standing heat. The amplitude of peak FSH concentration (ng/ml) and interval (h) between estrous onset to peak FSH did not differ significantly in animals with (14.8±1.2, 51.0±16.5) or without (15.8±0.6, 33.6±8.7) standing heat. A definite temporal coupling between LH and FSH secretions was absent.

The results suggest 1) frequent short-duration low-amplitude LH and FSH surges may be important for the final maturation of ovulatory follicle and subsequent ovulation; 2) differential regulatory mechanism probably exists to control the LH and FSH secretions.
RETINOIC ACID (RA) REGULATION OF TYPE II RECEPTORS FOR THE TGF-β SUPERFAMILY IN A MOUSE ADRENOCORTICAL CELL LINE

Y. Wang, J. K. Findlay, P. G. Farnsworth

Prince Henry’s Institute of Medical Research, Clayton, VIC, Australia

Inhibin A binds its co-receptor, betaglycan, and the type II receptors for activin or bone morphogenetic protein (BMP) in high affinity complexes that block activin and BMP actions. We previously correlated changes in the levels of betaglycan mRNA induced by physiological factors in several mouse cell lines with changes in inhibin binding to betaglycan on the cell surfaces. However, RA increased the binding of inhibin A to mouse adrenocortical AC cells with little stimulation of betaglycan expression. The present studies were undertaken to examine possible explanations for this anomalous finding.

Treatment of AC cells with RA (30 µM) for 18 h increased total [125I]inhibin A binding to 124 ± 5% of the DMSO-treated control (mean ± SEM, n=12), whereas it suppressed the betaglycan mRNA level to 59 ± 7% of control (n=6), measured using real-time RT-PCR. RA treatment increased the [125I]inhibin A affinity labelling of a protein species of deduced size 75 kDa, consistent in size with several type II receptors for the TGF-β superfamily. AC cells were found to express mRNA encoding type II receptors for activin (ActRII, ActRIIB), BMP (BMPRII) and Mullerian Inhibiting Substance (MISRII), but expression of the type II receptor for transforming growth factor-β (TβRII) was minor. RA dose-dependently suppressed the levels of mRNA for ActRIIB and MISRII by around 50% in each case, and further decreased the low level of TβRII expression, while the level of mRNA for ActRII was little changed. However, the level of BMPRII mRNA in AC cells was increased more than 2.5-fold in response to RA (30 µM).

We conclude from these studies that the AC cells may respond to many TGF-β superfamily members, but not TGF-β, and that the BMP type II receptor is a candidate for the protein that mediates the increase in inhibin binding following stimulation of AC cells with RA.

Funded by the NH&MRC of Australia (RegKeys 241000 & 198705)

EFFECTS OF CORTICOTROPHIN RELEASING HORMONE AND SALBUTAMOL ON TERM PREGNANT HUMAN MYOMETRIAL CONTRACTILE ACTIVITY IN VITRO

E. K. Tyson, R. Smith, G. Angeli, M. Read

Mothers and Babies Research Centre, John Hunter Hospital, New Lambton Heights, NSW, Australia

The function of CRH in pregnancy is unknown. One potential role is modulation of myometrial contractility. CRH stimulation of myometrial cells/tissue in vitro increases cAMP suggesting it should cause uterine relaxation. These findings contrast with earlier studies which indicate that CRH augments the contractile effects of oxytocin1,2. Furthermore recent studies suggest that high levels of CRH, as found at term, can down-regulate CRH receptors3.

We hypothesized that high concentrations of CRH may lead not only to homologous desensitization of CRH receptors but also to heterologous desensitization of other receptors linked to adenylate cyclase/cAMP production such as the β2-adrenergic receptor.

To test this hypothesis, isometric tension recordings were performed on term, spontaneously contracting myometrial strips (n=8) obtained at Caesarean section. Strips (7 x 2 mm) were mounted in organ baths containing Krebs’ (37°C, pH 7.4, 95% O2/5% CO2). The effects of CRH 10−7 M alone and as pretreatment (for 30 mins) prior to salbutamol (10−10–10−6 M, log intervals) were examined. Responses were measured by integrating the area under the tension curve. Responses to CRH and salbutamol were compared as a percentage of spontaneous activity prior to treatment.

Salbutamol caused a 42±13% (mean±SEM) reduction in contractility, ranging between 13-100% relaxation. Pretreatment with CRH under the same conditions resulted in a 26±7% reduction in contractility, ranging from 4-60%. There was no significant difference in relaxation between salbutamol alone or with CRH. Treatment with CRH 10−7 M alone exerted no significant effects.

Conclusions: We did not show a significant difference between salbutamol alone or in the presence of CRH. However, there was significant interpatient variability in the responses to salbutamol, increasing the possibility of a type 2 error.

(1) Quartero et al. Placenta 10(5),1989,pp439
(3) Grammatopoulos et al. Molec Endocrinol 19(2),2005,pp474
A PREGNANCY WITH HYDATIDIFORM MOLE, CO-EXISTING TWIN AND SEVERE THYROTOXICOSIS: A CASE REPORT

T. Sivananthan¹, C. Perera¹,², D. Knox¹, R. Vaughan¹
¹Obstetrics and Gynaecology, Orange Base Hospital, Orange, NSW, Australia
²Orange Campus, University of Sydney, Orange, NSW, Australia

Hyperthyroidism in pregnancy occurs with a prevalence of 0.1-0.2%(1). Clinical hyperthyroidism is rarely described nowadays in patients with a molar pregnancy (2). The association of a live fetus and a co-existing mole within a twin pregnancy is an unusual event, occurring with a frequency of 1 per 22,000-100,000 pregnancies (3). We present the case of a 28-year-old woman with hydatidiform mole and co-existing twin. She presented to her general practitioner at 15 weeks gestation with a history of palpitations, tremor and weight loss of 5kg over a period of 3 weeks. Thyroid function tests showed a free T4 at 42.0pmol/L (8.0-22.0), Free T3 at 21.6pmol/L (2.5-6.0) and TSH at 0.009mU/L (0.3-4.0). Treatment was initiated with propylthiouracil (PTU). The serum β-hCG was noted to be grossly elevated at 3503219 IU/L at 17 weeks gestation (6000 – 50,000). Ultrasound evaluation demonstrated a normal fetus co-existing with a mass consistent with a hydatidiform mole at which point she was referred to the local base hospital. An endocrinology opinion was sought on admission and her PTU dose was reduced as she had marginally low thyroid function on testing. The pregnancy ended prematurely a few days later when pre-eclampsia developed and fetal death in-utero occurred at 23 weeks gestation. Following uterine evacuation clinical and biochemical resolution of the hyperthyroidism resulted with decline in serum β-hCG levels, enabling cessation of anti-thyroid treatment. She was later found to be thyroid receptor antibody negative. The fetus appeared morphologically normal. Histopathology of the mass confirmed a complete hydatidiform mole. We provide details of this distinctive case in which thyrotoxicosis was the initial presenting feature of the patient’s condition. A review of the literature indicates that despite numerous case reports of a complete mole with co-existing twin only a few report complicating hyperthyroidism.


SEX DIFFERENCES IN PLACENTAL CYTOKINE EXPRESSION AND THEIR RELATIONSHIP TO FETAL CORTISOL


Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

In human pregnancy, there are sexually dimorphic differences in morbidity and mortality with the male fetus being more at risk of a poor outcome than the female fetus in association with complications such as placental insufficiency, pre-eclampsia, infection, intra-uterine growth restriction and pre-term delivery. The physiological mechanisms that confer differences in mortality in human male and female fetuses are unknown. The hypothalamic-pituitary-adrenal (HPA) axis and immune system are closely linked as adrenal glucocorticoid production modulates the inflammatory response by inhibiting pro-inflammatory cytokine mRNA expression. Sex specific differences in HPA and immune function have previously been observed in neonatal animal models and adult humans due to differences in cortisol bioavailability. In the human placenta, we have identified that sexually dimorphic cortisol bioavailability is controlled by 11β-hydroxysteroid dehydrogenase type 2 (11b-HSD 2) activity and this may confer differences in fetal-placental immune function. We hypothesise that there are sex specific differences in placental cytokine expression due to differences in cortisol bioavailability. In this study we aim to determine if there are sex specific differences in human placental cytokine mRNA expression and whether this is related to cord blood cortisol concentrations. Fetal cord blood was collected at the time of delivery and cortisol levels were measured using a commercial radioimmunoassay. Quantitative Real Time RT-PCR was used to examine the expression of cytokines in the placenta. The expression of cytokine mRNA in males (n=10) was positively correlated to the concentration of cortisol in cord blood. This occurred for TNFα (R²=0.444, P=0.0255), IL-6 (R²=0.4984, P=0.0289), IL-1 (R²=0.5117, P=0.0310), IL-8 (R²=0.6745, P=0.0038), and IL-5 (R²=0.4524, P=0.0331). There was no correlation between cortisol and female (n=11) placental cytokine mRNA expression. This data suggests there is a sexually dimorphic difference in the relationship between cortisol and placental cytokine expression.
MATERNAL AND CORD PLASMA CYTOKINE / CHEMOKINE PROFILES IN PREGNANCIES COMPlicated BY ASTHMA

A. Osei-Kumah¹, A. J. Ammit², R. Smith¹, V. L. Clifton¹

¹Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia
²Faculty of Pharmacy, University of Sydney, Sydney, NSW, Australia

The influence of pregnancy on maternal asthma is not unimodal but studies have indicated that in about a third of patients, asthma worsens as gestation progresses particularly in the last trimester. The reason for this effect is unknown. Asthma is an inflammatory disease characterised by the activation of T helper (Th) -2 response and expression of chemokines promoting the recruitment and survival of activated immune cells such as T-lymphocytes, eosinophils and neutrophils. In addition pregnancy is also associated with a Th-2 response. The aim of this study was to determine whether inflammatory mediators interleukin (IL) -6, IL-8, eotaxin and regulated upon activation normal T cell expressed and secreted (RANTES) are increased in pregnancies complicated by asthma. Peripheral blood was collected from asthmatic (n = 35) and nonasthmatic patients (n = 13) in the third trimester (30-32 weeks) of pregnancy. Cord blood (n = 24) was also collected after normal vaginal delivery. Blood samples were centrifuged at 1000g for 15 minutes and plasma samples were collected and assayed for IL-6, IL-8, eotaxin and RANTES using an enzyme linked immunosorbent assay (ELISA) kit. The levels of all the cytokines measured were significantly lower in the maternal circulation compared with the cord plasma (Kruskal Wallis, P < 0.01) in both asthmatic and nonasthmatic patients. There were no significant differences in the levels of maternal IL-6, IL-8, eotaxin and RANTES between asthmatics and nonasthmatics, though there was a trend towards increased level in asthmatic individuals. Interestingly, eotaxin was the only asthma specific chemokine that was significantly higher in cord plasma of asthmatic patients (Mann-Whitney, P = 0.004) compared with cord plasma from normal pregnancies. The results of this study suggest that eotaxin production is increased in the feto-placental unit of asthmatic pregnancies. The presence of asthma does not enhance Th-2 cytokine production during pregnancy.

IDENTIFICATION AND CHARACTERISATION OF MACROPHAGES IN PLACENTAE FROM PREGNANCIES COMPlicated BY ASTHMA

H. J. Wyper, K. G. Roberts, M. Delahunty, V. L. Clifton

Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

Maternal asthma during pregnancy has been associated with poor foetal outcome. Further studies have demonstrated that the outcome of pregnancy in optimally treated asthmatic women with inhaled glucocorticoids does not significantly differ from that of healthy women. Prospective studies reveal gender-specific outcomes in pregnancies complicated by asthma. In the presence of a female foetus, studies have observed the exacerbation of maternal asthma and an increased requirement for treatment of symptoms. It has also been identified that when asthma, regardless of its severity, is not treated during pregnancy with inhaled steroids female foetal growth is significantly reduced. The male foetus appears unaffected by asthma or its treatment. The increased inflammation evident with asthma is thought to alter placental function. Studies have noted an increase during gestation of monocytes in the maternal circulation in asthmatic women who did not use inhaled steroids and were pregnant with a female foetus.

Since monocytes are the precursors to macrophages and increased recruitment of placental macrophages has been associated with poor pregnancy outcome, this study aimed to identify and quantify the population densities of placental macrophages. Immunohistochemistry was utilised with specific staining of macrophages using CD68, to assess the effect of asthma on these inflammatory cells. Populations were also assessed in relation to glucocorticoid treatment and the presence of a female or male foetus, with these assessments compared to a non-asthmatic control group. No significant difference was observed between the placental macophage number in pregnancies complicated by asthma, with (n=50) or without glucocorticoid treatment (n=50), and non-asthmatic pregnancies (n=39). In addition, there was no evidence of a gender-specific difference in the placental macrophage population. This study demonstrates that the poor foetal outcome associated with pregnancies complicated by asthma may not be due to increased infiltration of placental macrophages.
PLACENTAL GLUCOCORTICOID RECEPTOR EXPRESSION IN PREGNANCIES COMPLICATED BY ASTHMA


Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia

Foetal growth and neonatal birth weight are significant contributing factors to the development of adult disease states in later life. Most animal models indicate that glucocorticoids are central in the regulation of organ maturation and foetal growth with increased bioactive cortisol being associated with reduced foetal growth. In human pregnancy, we have identified sexually dimorphic differences in foetal growth with the female foetus reducing growth in response to maternal asthma and the male foetus continuing to grow at a normal rate but being at an increased risk of in utero death. Interestingly, both the male and female foetuses were exposed to the same concentration of cortisol and yet there was a differential growth response. These findings lead to the question of whether there were sexually dimorphic differences of glucocorticoid receptor expression in placenta of male and female foetuses in pregnancies complicated by asthma that may confer differences in the response to cortisol. Both total GR and the alpha isoform mRNA expression were measured using quantitative RT-PCR in asthmatic and non-asthmatic pregnancies. GR total and GRα mRNA expression was significantly greater in placenta of female foetuses (n=6) from non-asthmatic pregnancies when compared to placentae of males (n=10). In the presence of asthma, GR mRNA was decreased in placentae of female foetuses (n=16) and increased in the placentae of male foetuses (n=21). These findings indicate that the sexually dimorphic response to a change in cortisol concentration may be mediated by differences in GR expression and regulation.

DIABETIC PREGNANCY & CONGENITAL MALFORMATIONS: WHEN SHOULD WE STOP WORRYING?

C. R. Ong, G. Ross

Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

Pregnancy outcomes for women with pregestational diabetes in the last decade have been disappointing despite the targets set by the St Vincent’s Declaration in 1989. It has been well established that congenital malformations are strongly associated with poor metabolic control prior to and during the pregnancy. A recent Australian audit in 10 teaching hospitals with an interest in high risk obstetric medicine demonstrated a four times increase in the stillbirth and congenital malformation rate, similar to the findings in reported studies from Europe, UK and New Zealand. Of the major malformations, congenital heart disease is one of the commonest. Fetal echocardiogram is currently recommended in pregestational diabetes in the early second trimester. There is a persistent lack of awareness about the need for pre-conception planning, tight maternal diabetes control and antenatal screening. We report 2 cases that highlight these important issues.

Case 1: A 38 year old woman with type 1 diabetes who had suboptimal glycaemic control prior to and during her pregnancy. Tight control was limited by frequent and unexpected episodes of hypoglycaemia. Antenatal fetal structural and growth ultrasound scans were normal although a fetal echocardiogram was not performed. A subsequent neonatal echocardiogram revealed a ventricular septal defect with pulmonary regurgitation requiring surgical correction.

Case 2: A 34 year old woman with type 1 diabetes had an unplanned pregnancy and poor metabolic control in the first trimester. Fetal echocardiogram at 24 weeks gestation was normal. Postnatal paediatric echocardiogram demonstrated moderate pulmonary stenosis and atrial septal defect with left to right shunting. This was managed conservatively with close monitoring.

A literature review and a local audit currently in process will be discussed.
ONCOGENIC OSTEOMALACIA: A CASE OF DIAGNOSTIC DILEMMA AND MANAGEMENT CHALLENGE
C. R. Ong¹, R. Mansberg², J. Bleasel³, M. Hooper¹
¹Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia
²Department of Nuclear Medicine, Royal Prince Alfred Hospital, Camperdown, NSW, Australia
³Department of Rheumatology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

A 75 year old previously independent woman presents with a significant decline in mobility preceeded by progressive proximal myalgia and weakness. Investigations revealed multiple minimal trauma fractures, marked renal phosphate wasting and apparent impaired vitamin D metabolism consistent with oncocenic osteomalacia. Diagnosis was further confirmed by a bone biopsy with tetracycline label and an elevated fibroblast growth factor 23 (FGF-23) level. Imaging studies demonstrated a left adrenal mass, left superior parathyroid adenoma and left thyroid nodule, all of which were octreotide avid. The patient responded well to vitamin D and phosphate supplements. A clinical decision was made to have all 3 lesions surgically excised. Pathology revealed benign adenoma of both the adrenal and parathyroid mass. However, papillary carcinoma with capsular invasion was demonstrated in the hemithyroid specimen. FGF-23 levels fell postoperatively but rose again in 3 months, coinciding with a clinical recurrence upon cessation of the supplements. Review of the MRI scans raised suspicion of multiple hemangiommas in a number of thoracic vertebrae and an unusual lesion anterior to the mandible. Selective venous sampling and positron emission tomography are currently considered. Oncogenic osteomalacia is an acquired paraneoplastic syndrome. Traditionally, there is a long lag time to diagnosis and location of culprit lesions. Phosphatonin proteins are proteins produced by the responsible tumour. Fibroblast growth factor 23 (FGF-23) has been demonstrated to be a major phosphatonin and other candidates have also been discussed in literature. Thyroid carcinoma has not been reported in literature to be associated with oncogenic osteomalacia but the findings in this patient may be coincidental. Whilst oncogenic osteomalacia is a rare disease, it is important to consider it in the assessment of patients presenting with weakness and pain. A literature review of novel therapies will be discussed.

CASE PRESENTATION: WIDESPREAD OSTEITIS FIBROSA CYSTICA AND PRIMARY HYPERPARATHYROIDISM IN A 41 YEAR OLD WOMAN.
M. Lewis, J. Carter
Department of Endocrinology and Metabolism, Concord Repatriation Hospital, Concord, NSW, Australia

Osteitis fibrosa cystica (OFC) is a rare complication of primary hyperparathyroidism. We present the case of a 41 year old Sri Lankan lady who presented with atraumatic fracture other right ulnar associated with hypercalcaemia up to 4.22mmol/l (ref range 2.15-2.55), X-rays revealed multiple lytic lesions throughout her long bone, pelvis, ribs and skull consistent with OFC. Subsequent investigations confirmed diagnosis of primary hyperparathyroidism with an elevated PTH of 140 pmol/l (ref range 1.6-7), a low 25-OH vitamin D 21nmol/l (ref range 31-107) and a bone specific ALP of 95.9ug/l (ref range 2.9-14.5). Bone scan revealed multiple "hot" lesions throughout her skeleton and a sesamibi scan revealed uptake in the mediastinum, due to an intrathymic parathyroid tumour measuring 4.5 x 3.5x 1.5cm. Representative radiology, bone scans and parathyroid imaging will be shown.

Therapy instituted to try to prevent the post operative occurrence of the “hungry bone syndrome” (including pre-operative calcitriol) will be described. Her post operative course, with respect to calcium, phosphate, Vitamin D, bone mineral density and radiology will be described in detail. Theories as to why OFC is rare will be discussed.

In summary she had a dramatic improvement in her plasma calcium, 24hour urinary calcium excretion initially, and with follow up until the time of the meeting we hope to show improvement in her radiology and BMD.

2. Rao et al, JBMR 2002
3. Harinarayan, Clin Endo 1995
THE MONITORING OF VITAMIN D IN OSTEIN-TREATED PATIENTS
A. McCormack, B. Luttrell, P. Clifton-Bligh, A. McElduff, J. Monaghan, G. Fulcher
Endocrinology, Royal North Shore Hospital, St Leonards, NSW, Australia

The measurement of serum 25-hydroxyvitamin D (25-OHD) has become routine in our unit as part of the investigation and management of patients with low bone density. Our usual practice is to titrate the dose of replacement vitamin D2 (Ostein 1000IU) to achieve blood levels of 25-OHD in the middle of the normal range. Recently we observed that despite increasing oral doses of Ostein, serum 25-OHD levels failed to rise as expected. Literature reports have called attention to inconsistencies in specificity and standardisation of assays for 25-OHD (1) and accordingly we questioned whether our current automated method (the competitive protein binding method (CPB)) run on the Advantage® analyser (Nichols Institute Diagnostics)), was appropriate for patients supplemented with vitamin D2. To assess this, we compared the results using the Nichols Advantage® Autoanalyser to those obtained using a manual RIA method (Diasorin) in a cross-sectional study of patients receiving Ostein (Vit D2) supplements. Baseline values from a group of untreated patients (who were not vitamin D deficient) were also included. Mean 25-OHD levels are shown in the table.

<table>
<thead>
<tr>
<th>Ostelin Dose</th>
<th>Nichols CPB (nmol/L)</th>
<th>Diasorin RIA</th>
<th>Significance (RIA vs CPB)</th>
<th>RIA:CPB Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (n=72)</td>
<td>68.3</td>
<td>73.4</td>
<td>ns</td>
<td>1.12</td>
</tr>
<tr>
<td>10000IU/d (n=62)</td>
<td>53.0</td>
<td>72.2</td>
<td>p&lt;0.0001</td>
<td>1.44**</td>
</tr>
<tr>
<td>20000IU/d (n=40)</td>
<td>48.0</td>
<td>78.8</td>
<td>p&lt;0.0001</td>
<td>1.68**</td>
</tr>
<tr>
<td>30000IU/d (n=6)</td>
<td>44.2</td>
<td>62.2</td>
<td>ns</td>
<td>1.53</td>
</tr>
<tr>
<td>40000IU/d (n=2)</td>
<td>36.9</td>
<td>74.0</td>
<td>ns</td>
<td>2.01</td>
</tr>
</tbody>
</table>

** Highly significant (p<0.0001) for mean ratio (vs. untreated). At higher doses the significance was lost possibly due to small sample sizes.

For patients naive to supplementation, the RIA and CPB assays produced similar measures of 25-OHD. However, their ratio was increased for each of the dosage groups, possibly reflecting the relative underestimation by the Nichols Advantage® of an increased proportion of 25-OHD2 present in the serum.

Conclusion: The Nichols CPB method appears to be underestimating 25-OHD2 in patients receiving vitamin D2 replacement. In its present form, it may not be suitable for monitoring vitamin D sufficiency when vitamin D2 is prescribed.


IMPACT OF A HOSPITAL-BASED INTERVENTION ON THE OUTCOME OF MINIMAL TRAUMA FRACTURES
S. Elkassaby, K. Quick, C. P. Gilfillan
Endocrinology, Peninsula Health Care Network, Frankston, VIC, Australia

Women who sustain a low trauma fracture are at significantly increased risk of subsequent fracture, and suffer increased morbidity and mortality. A number of medical therapies have now been proven in randomized controlled trials to reduce the incidence of minimal trauma fracture in selected high-risk women. Despite this, most minimal trauma fracture patients are discharged from hospital without the initiation of effective medical therapy to prevent recurrent fractures. Public healthcare institutions have an obligation to act to close this “care gap”. This study is a prospective randomised evaluation of the efficacy of a hospital-based intervention delivered to patients admitted with a minimal-trauma fracture at our institution. Patients with a diagnosis of a fragility fracture were randomised to two groups: an intervention group and a conventional treatment group. The intervention consisted of: clinical review, dual energy X-ray absorptiometry (DEXA) scan, basic biochemistry, a letter of recommendation to the LMO, provision of educational materials and if appropriate initiation of Calcium and Vitamin D, a Bisphosphonate or Raloxifene. The conventional group had no specific intervention and were managed by orthopaedic and rehabilitation specialists. Both groups were followed up (and will continue to be followed up at 1 and 2 years) to determine the proportion of patients who are initiated and remain on effective anti-fracture therapy (primary outcome). At this stage, baseline data for this study is available: 112 patients have been enrolled: 56 in intervention group, 56 in standard-care group. 12 patients have completed the biochemistry, DEXA and clinic review. Of these results are as follows: Biochemistry – one male with mild testosterone deficiency; one case of subclinical hyperthyroidism. All had 25(OH) Vitamin D3 < 100. Average level was 30-40. Skeletal risk score – three patients with a score between 2 and 5 with the remainder <1. DEXA scan: One patient with osteoporotic range T scores. 2 patients with T<-1.5 (at Femoral Neck T score (-1.66 and -2.02) Lumbar spine (-1.7 and -1.83) All except 2 patients had a T score < -1 at both femoral neck and lumbar spine. Treatment initiated: Risedronate on one patient, Vitamin D initiated on 2 patients. The data will be updated prior to presentation to present a more complete overview of our baseline population.
ASSESSMENT OF THE CLINICAL UTILITY OF URINARY NTX IN OSTEOPOROSIS—AN AUDIT
M. J. Gillett, S. D. Vasikaran
Core Clinical Pathology and Biochemistry, Royal Perth Hospital, Perth, WA, Australia

Background: Urinary levels of cross-linked N-terminal telopeptide of type I collagen (NTX) are used as a marker of bone resorption and are useful for the diagnosis of metabolic bone diseases and monitoring response of patients treated with antiresorptive agents. We aimed to determine how urinary NTX results alter clinical decision making by physicians treating patients with osteoporosis in a tertiary hospital setting.

Methods: we reviewed patient notes of all new NTX requests in 2002 and 2003 with at least one subsequent repeat measurement. Patients with a diagnosis of osteoporosis and both pre- and post-treatment measurements of bone mineral density (BMD) and NTX were included. Urinary NTX was measured with the Osteomark enzyme-linked immunosorbent assay (Wampole Laboratories, Princeton NJ, USA). BMD of the hip and lumbar spine was measured using dual energy x-ray (DEXA).

Results: A total of 357 patients had serial NTX requests during the time period. Sixty five of these patients had a diagnosis of osteoporosis. Out of 37 patients treated for osteoporosis who had complete data available, 29 patients had concordant results between BMD and NTX and 8 patients had discordant results. Of these only one patient had treatment changed as a result of a lack of reduction in NTX following treatment. Thirteen patients had therapy altered. Common reasons for altering therapy were patient non-compliance, side effects and failure of BMD to increase.

Conclusions: Alteration to therapy in this patient population is mainly dictated by issues such as patient compliance, medication side effects and bone mineral density results rather than urinary NTX values.

CARDIOVASCULAR EFFECTS OF MEDICAL THERAPIES IN POLYCYSTIC Ovary SYNDROME
C. Meyer, B. P. McGrath, D. Kotsopoulos, H. J. Teede
Medicine, Dandenong Hospital, Dandenong, VIC, Australia

Aim: To determine the impact of commonly used treatments for PCOS on CV risk factors including IR and on non invasive surrogate markers of CV disease.

Methods: 100 overweight women with PCOS were randomly assigned to either 35mcg ethinyl estradiol (EE)/2mg cyproterone acetate (CPA), Metformin (1g twice daily) or a low dose OCP (20mcg EE/100mcg levonorgestrel) combined with an Aldactone 50mg bd for 6 months. Arterial structure and function and metabolic parameters were assessed before and 6 months after treatment. Arterial structure and function was assessed using carotid intimal media thickness [IMT], pulse wave velocity [PWV] and flow mediated vasodilation [FMD]. Metabolic parameters assessed included insulin and glucose during an OGTT, lipid parameters and serum androgens.

Results: All 3 treatments significantly improved hirsutism and menstrual cycle length however only the OCP groups showed improvements in androgens. IR improved in the Metformin group and deteriorated in the higher dose OCP group. Arterial stiffness worsened in the higher dose OCP group (PWV 7.46 vs. 8.03 m/s P<0.05). The increase in IR was a significant predictor of the increased arterial stiffness.

Conclusions: In overweight women with PCOS Metformin, the low dose OCP/Aldactone combination and a higher dose OCP had similar clinical efficacy. Metformin decreased IR, the low dose OCP had a neutral effect but the high dose OCP was associated with worsening IR and arterial stiffness. If an OCP is required by women with PCOS a low dose estrogen preparation should be used. Insulin sensitizing agents should be considered as the primary therapy in the symptomatic management of women with PCOS particularly in those with additional CV risk factors.
CLINICAL EXPERIENCE WITH 2 YEARS OF Zoledronic Acid IN OSTEOPOROSIS

D. Hughes¹, B. White², B. Crawford¹,²,³, M. Hooper¹,²,³

¹The University of Sydney, NSW, Australia
²Endocrinology, Concord Hospital, NSW, Australia
³Endocrinology, RPAH, NSW, Australia

Intravenous (IV) zoledronic acid (ZA; Zometa, Novartis Pharmaceutical) therapy is used in the management of osteoporosis (Reid et al. 2002). In this study we report our experience with ZA in routine clinical practice in regard to effect on bone mineral density (BMD) in osteoporosis. We reviewed the records of patients who had been given IV ZA (4mg) as a single annual dose. BMD was routinely measured on the day of the first, second and third annual infusion. Results were available at 0 and 1 years (Group 1) in 55 patients (36 women and 14 men), with a mean age of 68 years (range, 19-92 years) and 0, 1 and 2 years in a subset of 39 patients (all postmenopausal women) (Group 2). The analyses used paired t tests. Results are mean ± SEM. In group 1, lumbar spine BMD was increased significantly (3.8 ± 0.7%) at 12 months (P<0.0001), femoral neck also increased significantly (2.0 ± 0.5%) at 12 months (P=0.0001). In group 2, who were followed for a further 12 months, the change in BMD compared to baseline were at the lumbar spine +3.1 ± 0.7% and +4.3 ± 0.9% respectively at 1 and 2 years, and at the femoral neck 2.5 ± 0.6% and 2.5 ± 0.8%. No new incident fractures were observed.

(1) Reid, IR et al. NEJM 2002; 346:653-661

THyroTOXIC hyPOKALaEMIC PERIODIC PARALYSIS IN A YOUNG AUSTRALIAN CAUCASIAN MAN: A CASE REPORT

C. Perera¹,², A. Shearer³

¹Greater Western Area Health Service, Orange Base Hospital, Orange, NSW, Australia
²Orange Campus, University of Sydney, Orange, NSW, Australia
³Cairns Base Hospital, Cairns, QLD, Australia

A 23-year-old Australian Caucasian male presented to a country hospital with rapid onset quadriplegicis with a several weeks' history of palpitations increased anxiety, tremors and tiredness suggestive of thyrotoxicosis. Examination revealed tachycardia, hypotonia, and quadriplegicis. He was haemodynamically stable. Neck examination showed a smooth goiter with a loud Thyroid bruit and he was positive for thyrotoxic eye signs.

On admission his investigation results were as follows. Serum Potassium = 1.7 mmol/L (3.5-5.6), TSH = 0.0 uU/ml, FT4 = 69.9 pmol/L, FT3 > 35 pmol/L, ESR = 15, Cr = 44 umol/L, Urea = 4.1 mmol/L, Thyroid receptor Antibodies = 14.9 IU/L, TC99 thyroid uptake scan showed a homogeneous diffuse uptake of 17%. His family history was unremarkable for thyroid disease or periodic paralysis.

He was treated with intravenous and oral potassium supplementation, Carbimazole and Propranolol. Following correction of his hypokalaemia his quadriplegicis improved rapidly and he walked out of hospital upon discharge two days later on Carbimazole and Propranolol therapy. For the last twelve months he has maintained euthyroidism on a small dose of Carbimazole therapy with out any further episodes of thyrotoxicosis or periodic paralysis.

Thyrotoxic Hypokalaemic Periodic Paralysis is relatively common amongst Asians and Latin Americans but remain very rare amongst the Caucasians. A total of about twenty cases are reported in the literature and only a single case was previously published of a white Australian with this condition(1).

(1) Sivaganganbalan G et al., Internal Medicine Journal 2003 Sep-Oct; 33(9-10):475
INSULIN LIKE GROWTH FACTOR-2 OCCUPANCY OF THE INSULIN RECEPTOR ISOFORM A: SIGNIFICANCE IN NORMAL AND MALIGNANT TISSUES.
B. P. Shelton, V. F. Bailey, L. Xie, E. Helmerhorst

Western Australian Biomedical Research Institute, Curtin University of Technology, Bentley, WA, Australia

Insulin and insulin-like growth factor-2 (IGF-2) play disparate roles in adult tissue. While insulin is predominantly a metabolic hormone, IGF-2 plays a significant role in promoting cell growth. Despite this, recent results have suggested that an alternatively spliced isoform of the insulin receptor missing exon 11 (IR-A) binds both insulin and IGF-2 with high affinity. Although both hormones can activate IR-A to produce their specific effects, the full-length insulin receptor, IR-B, is apparently specific only for insulin. Expression of these isoforms is highly tissue-specific. However, it has never been determined if the IGF-2 preference for IR-A correlates with increased IGF-2 occupancy of the insulin receptor, and thus increased signaling. In this study, we investigated the affinity and occupancy of insulin and IGF-2 for insulin receptors purified from rat liver and brain, which express predominantly IR-B and IR-A, respectively. These calculations revealed that, in tissues expressing predominantly IR-B, insulin receptors are 41% and 3% occupied by insulin and IGF-2, respectively. However, in tissues expressing predominantly IR-A, insulin occupancy of receptors increased marginally to 49%, while IGF-II occupancy increased significantly to 16%. However, in the brain where IR-A is also predominant, insulin receptors were 93% and 1% occupied by insulin and IGF-II, respectively. These results suggest that IGF-2 signaling by IR-B, or by IR-A in the brain, play only a minor role. However, IGF-2 signaling in other tissues predominantly expressing IR-A, likely plays a significant role, especially in some malignant tissues that secrete IGF-2 and over-express IR-A.

withdrawn

THE MULTIFUNCTIONAL PROTEIN CREAP IS A NUCLEAR PROTEIN
K. L. Shipman, R. Smith, R. C. Nicholson

Mothers and Babies Research Centre, Hunter Medical Research Institute, University of Newcastle, Newcastle, NSW, Australia

The cAMP regulatory element (CRE) is one of the most important regulatory elements determining up regulation of corticotropin releasing hormone (CRH) in the placenta and hypothalamus. A placental cDNA library has previously been screened using the yeast one-hybrid system to identify proteins capable of functionally binding the CRE. A human cDNA encoding a protein with a distinctive combination of modular domains was discovered. This new protein has been named CRE Associated Protein 1 (CREAP1). CREAP contains two leucine-zipper-like domains typical of bZIP transcription factors, a zinc finger-like domain with potential DNA binding properties, a zinc finger-like domain typical of RNA binding proteins, two coiled-coil domains typically found in transcription factors and an SR-rich domain characteristic of proteins involved in RNA splicing.

CREAP has been shown to specifically bind to the CRE of the CRH promoter using an electrophoretic mobility shift assay. A multiple tissue expression array has shown that CREAP is present in a wide variety of human adult and fetal tissues. The CREAP peptide sequence was compared to the protein databases and two highly related proteins of unknown function were found. They are 95% similar to each other over the N-terminal two-thirds and are all very similar (60%) to CREAP1. All three proteins share the coiled-coil, zinc finger, leucine zipper and SR domains. These protein sequence and domain similarities suggest that a new family of human proteins uniquely capable of binding to the CRE and to function in RNA splicing has been identified.

Western blotting has shown that CREAP is only detected in nuclear or total protein extracts and not in cytosolic protein extracts. This nuclear localization further supports a role for CREAP as a transcription factor and/or splicing protein.
IDENTIFICATION OF RENIN AND REGULATORY ROLE OF THE RENIN mRNA-BINDING PROTEINS HUR, HADHB AND CP1 IN HUMAN BREAST CANCER CELLS.

L. E. C. Miles, D. J. Beveridge, H. Mangs, H. Speirs, B. J. Morris, P. J. Leedman

1Laboratory for Cancer Medicine, Centre for Medical Research, Western Australian Institute for Medical Research, The University of Western Aus, Perth, WA, Australia
2Basic and Clinical Genomics Laboratory, School of Medical Sciences and Institute for Biomedical Research, The University, Sydney, NSW, Australia

Renin is the enzyme responsible for the cleavage of angiotensinogen to produce angiotensin I and forms a key part of the renin-angiotensin system (RAS). This system is not only crucial for the regulation of blood pressure, fluid and electrolyte balance, but can also affect both cell proliferation and apoptosis. Although renin is typically expressed in the juxtaglomerular cells of the kidney, it has now also been detected in extra-renal sites such as the pancreas, and in the renin-expressing pulmonary carcinoma cell line Calu-6, suggesting renin may be more widely expressed than was once perceived. Of interest, preliminary results from our laboratory have identified (via RT-PCR) expression of renin mRNA in both MDA-MB-468 and MCF-7 breast cancer cells. To our knowledge, this is the first evidence of renin expression in breast cancer cells. We have previously demonstrated (1) that the RNA-binding proteins (RBPs) HuR, HADHB and CP1 bind to the renin 3'UTR and that both renin mRNA and protein levels can be regulated by these RBPs. As such, we wished to determine whether the renin mRNA and protein detected in two breast cancer cell lines was regulated by these RBPs. Immunoprecipitation-RT-PCR and GST pulldown assays are being utilised to assess whether these RBPs immunoprecipitate and interact with renin mRNA in MDA-MB-468 and MCF-7 breast cancer cell lines. In addition, we have utilised siRNA and Western blotting to determine the effect of reducing endogenous RBP levels on renin mRNA and protein expression, while also examining these cells for any resultant effects on proliferation. Our results to date indicate that reducing the levels of HuR significantly reduces renin expression, suggesting a key role for this protein in the renin expression and signaling pathway. In sum, our data demonstrate renin mRNA for the first time in breast cancer cells, and that renin expression is likely to be governed, at least in part, by renin RBPs such as HuR. Further studies are underway to explore the effect of these RNA-protein interactions on breast cancer cell growth and proliferation.


EXPRESSION OF SFRP-4 AND β-CATENIN IN SEROUS OVARIAN CARCINOMA

J. Drake, A. Shearwood, N. Zeps, A. Dharmarajan

1School of Anatomy and Human Biology, The University of Western Australia, Crawley, WA, Australia
2School of Surgery and Pathology, The University of Western Australia, Crawley, WA, Australia

Ovarian cancers may arise from mutations that activate the Wnt signalling pathway, as elevated Wnt expression has previously been demonstrated in ovarian cancer. Secreted frizzled-related proteins (sFRPs) comprise a family of five secreted glycoproteins that antagonize Wnt signaling. Thus, a role for sFRPs as negative regulators of Wnt signalling may have important implications in ovarian tumorigenesis. In the present study, we investigated the expression of sFRP-4, together with the downstream marker of Wnt activation, phosphorylated β-catenin, in 163 serous ovarian adenocarcinoma samples by using tissue microarrays (TMA) and immunohistochemistry (IHC). Ovarian cancer samples expressed higher levels of sFRP-4 compared to adjacent normal stroma. However, sFRP-4 expression was not correlated with any clinico-pathological features such as age, International Federation of Gynecological Oncologists (FIGO) stage or histological grade. Therefore, sFRP-4 expression was not an independent prognostic marker for serous carcinoma of the ovary. In addition, FIGO stage was significantly correlated with patient survival (p<0.01) but there was no significant relationship between histological grade and survival.

A trend towards improved survival was observed in patients whose tumours exhibited high levels of sFRP4 staining. Therefore, this research may help to explain previous contradicting results from studies which examined sFRP4 as a prognostic marker in tumours of the prostate and colon. The proposal that sFRP4 may be used as a potential survival marker for prostate but not colon tumours could relate to the hormone-dependent nature of the tissue from which the cancer has derived. Expression of sFRP4 in these hormone dependent tumours suggests that sFRP4 could be over-expressed in order to reverse the uncontrolled cell proliferation.
TWO STRUCTURALLY-RELATED COUMARIN ANTIBIOTICS EXERT DIFFERENT EFFECTS ON DISRUPTION OF HSP90 DIMERIZATION

D. Mok, T. Ratajczak

Laboratory for Molecular Endocrinology, Western Australian Institute for Medical Research, Nedlands, WA, Australia

Heat shock protein 90 (Hsp90) is a molecular chaperone that is found in steroid receptor heterocomplexes and functions as a dimer, in association with p23 and one of four immunophilins, CyP40, FKBP51, FKBP52 or PP5, all of which are essential for promoting steroid hormone signalling. The C-terminal region of Hsp90 contains a site for chaperone function, a dimerization domain and a recognition site for the immunophilins. Also within this region is an overlapping binding site for coumarin antibiotics, such as novobiocin, which has been shown in our laboratory to interfere with binding to immunophilins. The binding site for novobiocin has been shown to be located within the dimerization domain and the aim of this project was to determine if novobiocin and another coumarin drug, coumermycin A_1, could disrupt Hsp90 dimerization.

Treatment with 0-20 mM novobiocin on a his-tagged Hsp90 C-terminal fragment (527-724) was assessed by native PAGE to determine the effect of the coumarin on dimerization. A dimer band was observed at all concentrations of novobiocin tested and under different temperature conditions, suggesting that novobiocin is unable to disrupt pre-formed Hsp90 dimers. Treatment with 0-1 mM coumermycin A_1, which is almost twice the size of novobiocin, on his-tagged Hsp90 527-724, was analysed by a chemical cross-linking assay to determine its effect on dimerization. Analysis by Western blotting revealed that the level of Hsp90 dimer decreased in the presence of coumermycin A_1 in a concentration-dependent manner. Native gel experiments using coumermycin A_1 are now underway to confirm these results. These data indicate that coumermycin A_1 is more potent at disrupting Hsp90 dimerization than novobiocin. In conclusion, our results suggest that the coumarin antibiotics antagonize Hsp90 function through a unique mode of action by destabilising Hsp90 dimerization.

THE HEAT SHOCK PROTEIN 90-BINDING COUMARIN NOVOBIOCIN INHIBITS STEROID RECEPTOR ACTIVITY WITHOUT THE STRESS

R. Allan\textsuperscript{1,2}, T. Ratajczak\textsuperscript{1,2}

\textsuperscript{1}\textit{Western Australian Institute for Medical Research and the UWA Centre for Medical, The University of Western Australia, Nedlands, WA, Australia}

\textsuperscript{2}\textit{Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia}

Heat shock protein 90 (Hsp90) is a molecular chaperone that regulates the stability of many key cell-signalling proteins such as steroid hormone receptors and protein kinases. Many of these signalling molecules are associated with cancer development and progression and thus Hsp90 presents as a unique anticancer target that influences many signalling pathways in tumour formation. C-terminal dimerisation of Hsp90 followed by the chaperone's ATPase activity are required for client protein activation in a multistep process. Hsp90 forms distinct heterocomplexes with steroid receptors and protein kinases by associating with tetratricopeptide repeat (TPR)-containing immunophilins or p50\textsuperscript{cdc37}, respectively. The antitumour agent cisplatin binds to the Hsp90 C-terminal domain and inhibits steroid receptor transcriptional activity through depletion of receptor protein. The coumarin antibiotic novobiocin has been shown to also bind to the Hsp90 C-terminal domain and cause depletion of the signalling kinaseserb2 and Raf-1, unlike cisplatin, which did not deplete kinases. Geldanamycin is a well-documented Hsp90 inhibitor that also causes signalling protein depletion but also induces a cellular stress response, which in the case of its derivative 17-AAG, causes osteoclast formation and subsequent bone destruction. Although cisplatin also impacted on steroid receptor activity, it was reported to not induce a stress response through the heat shock factor 1 transcription factor.

Previous findings in our laboratory have revealed that novobiocin is able to inhibit immunophilin and p50\textsuperscript{cdc37} association with Hsp90, but not disrupt Hsp90-immunophilin interaction. We have also observed decreased glucocorticoid receptor transcriptional activity in HeLa cells and now show that this reduced activity may be due to depleted receptor protein levels in the cells. Furthermore, like cisplatin, novobiocin was found to not induce a cellular stress response, as seen by monitoring Hsp70 protein production.