

16th Annual Scientific Meeting

Programme and Abstracts

21st November, 2004

Sheraton Hotel, Ching and Ming Rooms

Organizing Committee

Dr. Nelson M.S. Wat
Dr. Annette W.K. Tso
Dr. Alice S.T. Wong
Dr. Y.C. Woo

PROGRAMME

9:30 am – 10:00 am	Registration and Setting Up Posters		
10:00 am – 11:00 am	Scientific Oral Session Chairpersons: Dr. J. Luk & Dr. W.S. O		
10:00 am	S1	Gonadotropins: regulators of N-cadherin adhesion and inhibitors of cell survival in human ovarian surface epithelium	Y.L. Pon
10:15 am	S2	Transcription factors of the Sp1, CREB, and AP-1 families are important for nectin-2 gene transcription in testicular cells	K.L. Sze
10:30 am	S3	Grass Carp CREB: molecular cloning, functional characterization, and regulation of transcript expression by somatostatin in Carp pituitary cells	G. Fu
10:45 am	S4	Functional role of activin as a paracrine regulator of growth hormone gene expression in Grass Carp pituitary cells	R.S.K. Fung
11:00 am – 11:30 am	Tea Break and Poster Viewing (Clinical)		
11:30 am – 1:00 pm	Symposium Lectures Chairpersons: Prof. M. Leong & Prof. R. Young		
11:30 am		Issues concerning testosterone in Elderly Men	Prof. A.W.C. Kung
12:15 pm		Where are we with postmenopausal hormone therapy in 2003?	Dr. E.P.M. Lam
1:00 pm – 2:30 pm	Lunch and Poster Viewing		
2:30 pm – 3:30 pm	Clinical Oral Presentation Chairpersons: Dr. J. Ma & Dr. S.C. Siu		
2:30 pm	C1	Prevalence of osteoporosis and fractures in Chinese patients chronically treated with glucocorticoids	F.K.W. Chan
2:45 pm	C2	Genetic and clinical characteristics of maturity-onset diabetes of the young in Chinese patients	J.Y. Xu
3:00 pm	C3	Combined orbital irradiation with systemic steroid in comparison with systemic steroid alone in the management of severe Graves' ophthalmopathy	J.C.M. Ng
3:15 pm	C4	The effect of diet and orlistat on body weight and lipid profiles in Chinese patients with coronary artery disease (CAD), obesity and average to moderate hypercholesterolemia	K.W. Chan
3:30 pm – 4:15 pm	Tea Break and Poster Viewing (Scientific)		
4:15 pm – 4:30 pm	Award of Prizes for the Best Presentations		
4:30 pm – 5:15 pm	Annual General Meeting and Council Election		

ABSTRACTS

Symposium Lectures

- L1 **Kung AWC**
Issues concerning testosterone in Elderly Men
- L2 **Lam EPH**
Where are we with postmenopausal hormone therapy in 2003?

Oral Presentations: Scientific

- S1 **Pon YL, Wong AST**
Gonadotropins: regulators of N-cadherin adhesion and inhibitors of cell survival in human ovarian surface epithelium
- S2 **Sze KL, Lui WY, Lee WM**
Transcription factors of the Sp1, CREB, and AP-1 families are important for nectin-2 gene transcription in testicular cells
- S3 **Fu G, Ko WKW, Wong AOL**
Grass Carp CREB: Molecular cloning, functional characterization, and regulation of transcript expression by somatostatin in Carp pituitary cells
- S4 **Fung RSK, Ko WKW, Wong AOL**
Functional role of activin as a paracrine regulator of growth hormone gene expression in Grass Carp pituitary cells

Oral Presentations: Clinical

- C1 **Chan FKW, Tiu SC, Choi CH, Ng YW, Ng CM, Kong APS**
Prevalence of osteoporosis and fractures in Chinese patients chronically treated with glucocorticoids
- C2 **Xu JY, Dan QH, Chan V, Wat NMS, Tam S, Tiu SC, Lee KF, Siu SC, Tsang MW, Fung LM, Chan KW, Lam KSL**
Genetic and clinical characteristics of maturity-onset diabetes of the young in Chinese patients
- C3 **Ng JCM, Yuen HKL, Chan MK, Yuen KT, Leung DYL, Choi KL, Chan FKW, Choi CH, Ng YW, Tiu SC**
Combined orbital irradiation with systemic steroid in comparison with systemic steroid alone in the management of severe Graves' ophthalmopathy
- C4 **Chan KW, Leung WS, Fung YS, Hung HF, Tsui PT, Chu HK, Chan YW**
The effect of diet and orlistat on body weight and lipid profiles in Chinese patients with coronary artery disease (CAD), obesity and average to moderate hypercholesterolemia

Poster Presentations: Endocrinology and Metabolism

- P-C1 **Chow WS**, Tse HF, Tan KCB, Rui H, Lo KW, Lee KF, Lam KSL
Glycemic control and endothelial function in Chinese subjects with type 1 Diabetes Mellitus
- P-C2 **Tan KCB**, Shiu SWM, Wong Y, Tam S
Plasma phospholipids transfer protein activity and subclinical inflammation in type 2 Diabetes Mellitus
- P-C3 **Choi CH**, Tiu SC, Chan FKW, Ng YW, Ng CM
Reproducibility of bedside cardiovascular autonomic test in normal volunteers and type 2 diabetes
- P-C4 **Chan KW**, Xu A, Hoo RLC, Zhang JL, Lam MCW, Lam KSL
The role of testosterone in the determination of sexual dimorphism of adiponectin
- P-C5 Tiu SC, **Choi CH**, Shek CC, Ng YW, Chan FKW, Ng CM, Kong APS
The use of aldosterone-renin ratio (ARR) as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling
- P-C6 **Hung HFV**, Chan KW, Siu SMM, Wong YMB
An extended follow-up study of adrenocortical function of convalescent health-care workers who had received high dose steroids for the treatment of Severe Acute Respiratory Syndrome (SARS)
- P-C7 **NP Wong**, CC Chow, C Cockram, SC Siu
Endocrinological outcomes in patients with craniopharyngiomas in Hong Kong
- P-S1 **Wong AOL**, Huo L, Fu G, Ko WKW
Calmodulin as a novel mediator for feedback control of growth hormone gene expression by insulin-like growth factor in Grass Carp pituitary cells
- P-S2 **Wang X**, Ko WKW, Wong AOL
Dopaminergic regulation of growth hormone gene expression in Chinese Grass Carp via pituitary D1 receptors coupled to the cAMP/PKA, PI3K- and MAPK-dependent cascades

Poster Presentations: Reproduction

- P-S3 **Zhou HY**, Keung YK, Wong AST
High levels of Met expression in the control of hepatocyte growth factor-dependent invasive growth in ovarian cancer cells
- P-S4 He QY, Zhou Y, Wong E, Ehlen T, Auersperg N, Chiu JF, **Wong AST**
Proteomic analysis of human ovarian surface epithelial cells with germline mutations of BRCA1
- P-S5 **Lee KF**, Cheung WKL, Liu Y, Ng EHY, Ho PC
Expression profiling of endometrium from women with natural and hyperstimulated cycle during *in vitro* fertilization treatment
- P-S6 **Lui WY**, Lee WM
Ubiquitination of occludin in the regulation of tight junction barrier in the testis
- P-S7 **Au CL**, Yeung JL, Chung HS, Chan LF
Vascular endothelial growth factor and VEGF-related peptides in hCG-induced testicular angiogenesis
- P-S8 **Liao SB**, Chow PH, O WS
Leptin expression in embryos sired by male golden hamsters with all accessory sex glands removed
- P-S9 **Chung MK**, Krabsova L, Lau EYL, Ng EHY, Ho PC, Yeung WSB, Lee KF
Prevalence and the use of sY153 as marker of AZFd microdeletion in Chinese men

Poster Presentations (submissions not included in printed form)

- P-S10 Lee YL, Xu JS, Lee KF and **Yeung WSB**
A preliminary study on the role of leptin in signaling between early embryos and oviduct
- P-C8 **Ozaki R**, Qiao Q, Wong GWK, Chan M, So WY, Tong CY, Ho CS, Ko GTC, Lam CWK, Tuomilehto J, Chan JCN.
Prevalence of the metabolic syndrome in Hong Kong Chinese adolescents

ISSUES WITH TESTOSTERONE IN ELDERLY MEN

Annie W.C. Kung

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The enthusiasm in the use of testosterone has increased tremendously worldwide. In the US the number of testosterone prescription in 2003 was over 2 million, doubling that of 1999. Although no such figure is available for Hong Kong, testosterone therapy for elderly men to improve vitality, libido and mental acuity is gaining vast attention and popularity. Although short term studies documented certain beneficial effects of testosterone in elderly men, these studies are of small scale and short duration and the long term risks and benefits of testosterone therapy remain unaddressed.

Serum total testosterone concentrations decrease gradually with age, with about 20% of men over age 60 having values below normal for young men. However whether this decline is a physiological phenomenon to convey benefits or a pathological one causing harm remains unclear. To answer this question, short-term studies were performed to replace testosterone in hypogonadal elderly men. Overall the current scientific evidence suggests that testosterone therapy in elderly men is associated with improved body composition and possible bone mineral density and sexual function, but not with any other clinical parameters.

Another problem with testosterone therapy in older men is the lack of consensus for the cut-off level of serum testosterone that defines hypogonadism in older men. The diurnal variation of testosterone secretion and the poor precision of free testosterone assays hinder accurate diagnosis of hypogonadism. Patients should only be labeled as hypogonadal unless serum total testosterone is unequivocally low (<200ng/dl or 10nmol/l) on two occasions together with symptoms consistent with androgen deficiency.

The reason for potential concern for testosterone use in elderly men is that prostate cancer and benign prostatic hyperplasia, of which elderly men are prone to, are likely testosterone-dependent diseases. As many as 50% of men over the age of 50 may harbour occult prostate cancer. Also supraphysiological doses of testosterone are known to cause erythrocytosis and may increase sleep apnoea. However, none of the published clinical trials are large enough to address these long term risks. Thus until there are clear answers to these questions, testosterone therapy in older men should be considered carefully and patients should be adequately informed of potential risks of such treatment.

WHERE ARE WE WITH POSTMENOPAUSAL HORMONE THERAPY IN 2004?

Emily P.M. Lam

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Recently, two large randomized placebo-controlled studies on long-term postmenopausal hormone replacement therapy (HRT), the Heart and Estrogen/progestin Replacement Study (HERS) and the Women's Health Initiative trial (WHI), have raised a lot of controversies, especially on the cardiovascular aspects of HRT use. This lecture will briefly review these two trials and discuss what we know and do not know about HRT. It will also review the evidence on the new novel postmenopausal hormone therapy such as raloxifene and tibolone.

Both conventional HRT (estrogen \pm progestin) and tibolone can alleviate climacteric symptoms and may improve mood & sexual well-being while raloxifene has no such beneficial effect and may even increase the incidence of hot flush. All these therapies have been shown to increase bone mineral density in both the spine and the hip, as compared to placebo. However, only conventional HRT has been proven to prevent hip fractures. Raloxifene has been shown to reduce the incidence of vertebral fractures while there is limited information on tibolone for prevention of bone fractures. All these postmenopausal hormone therapies are associated with an increased risk of venous thromboembolism. There is also concern about conventional HRT on increased coronary events during the initial years of use. There is no evidence of any increased cardiovascular risk for both raloxifene & tibolone. Preliminary evidence showed that raloxifene reduced the incidence of the cardiovascular events by 40% in postmenopausal women with increased cardiovascular risk. In contrast to conventional HRT, both raloxifene & tibolone have no stimulatory effect on endometrium and have a high rate of amenorrhoea. Also, they have no breast stimulatory effect and have no increase in mastalgia or mammographic density.

GONADOTROPINS: REGULATORS OF N-CADHERIN ADHESION AND INHIBITORS OF CELL SURVIVAL IN HUMAN OVARIAN SURFACE EPITHELIUM

Yuen Lam Pon and Alice S.T. Wong
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Epithelial ovarian carcinoma is the most lethal gynaecological cancer and arises from the human ovarian surface epithelium (OSE), which is a single mesothelium covering the surface of the ovary. However, the normal reproductive physiology of OSE has not been fully disclosed. In this study, we report a new mechanism whereby gonadotropins, major regulators of ovarian function, regulate the survival of human OSE by down-regulating N-cadherin. N-cadherin is a major adhesion protein in human OSE our result indicated that disruption of N-cadherin mediated cell adhesion induced apoptosis of OSE. Here, we demonstrated that the expression of N-cadherin was under endogenous control by gonadotropins and this effect was mediated through cAMP/protein kinase A pathway, but not ERK1/2 and protein kinase C cascades. Furthermore, elevation of gonadotropins and intracellular levels of cAMP promoted the down-regulation of N-cadherin through proteasomal degradation suggesting expression of N-cadherin was regulated by a post-translational mechanism. Collectively, our results suggest regulation of N-cadherin is a critical event in controlling the survival capability of human OSE which may have an important role in normal ovarian physiology and ovarian carcinogenesis.

This work was supported by grants from the University of Hong Kong and the Hong Kong Research Grant Council.

TRANSCRIPTION FACTORS OF THE SP1, CREB, AND AP-1 FAMILIES ARE IMPORTANT FOR NECTIN-2 GENE TRANSCRIPTION IN TESTICULAR CELLS

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Nectin-2, a major protein component of the adherens junctions (AJs), is found between Sertoli cells and germ cells in the seminiferous epithelium. Recent studies have shown that the expression of nectin-2 gene in testis is crucial to maintain normal spermatogenesis since male knockout mice lacking nectin-2 gene are sterile and possess morphologically abnormal spermatozoa. However, the molecular mechanisms governing its basal transcription remain poorly understood. By the use of Sertoli and germ cell-lines (TM4 and GC-2 Spd (ts) cells respectively) in transient transfection studies, we showed that the minimal mouse nectin-2 promoter was located between nucleotides -316 and -211 (relative to the translation start site). Two putative Sp1 motifs and one each of the CRE, AP1 and AP2 motifs were identified within this region. Mutational studies showed that these two Sp1 motifs cooperated synergistically with the CRE motif, but not the AP1 and AP2 motifs, to regulate nectin-2 gene transcription in both TM4 and GC-2 Spd(ts) cells. By EMSAs, we found that an AP-1 consensus sequence was able to inhibit DNA-protein complex formation with the CRE/AP-1 motif, suggesting a cross-talk between the AP-1 transcription factor (c-Jun) and this CRE/AP-1 motif. Overexpression of CREB and a serine-133-mutated CREB also significantly increased the promoter activity, which suggests CREB to be one of crucial transcription factors involved in regulating nectin-2 gene transcription. It has been known that there is a cyclic expression of CREB throughout the spermatogenic cycle. Our present data provide an additional line of evidence that CREB regulates spermatogenesis and controls the timely assembly and disassembly of AJs between Sertoli cells and spermatids by regulating nectin-2 gene transcription.

This work was supported by grants from the University of Hong Kong and the Hong Kong Research Grant Council.

GRASS CARP CREB: MOLECULAR CLONING, FUNCTIONAL CHARACTERIZATION, AND REGULATION OF TRANSCRIPT EXPRESSION BY SOMATOSTATIN IN CARP PITUITARY CELLS

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CREB, a ubiquitous transcription factor, mediates the stimulatory effects of the AC/cAMP/ PKA pathway on gene transcription by binding directly to the CRE site(s) in the target gene promoter. Although functional CRE sites have been identified in the 5' promoter of growth hormone (GH) gene, pituitary expression of CREB and its role in neuroendocrine control of GH synthesis have not been previously examined. In this study, using grass carp as an animal model, we tested the hypothesis that somatostatin (SRIF), a GH-release inhibitor, could suppress GH gene expression by modulation of CREB expression at the pituitary cell level. As a first step, molecular cloning was performed to establish the structural identity of grass carp CREB using nested PCR coupled to 5'/3' RACE. The full-length cDNA obtained reveals that the a.a. sequence of carp CREB (324 a.a.) is highly homologous (86-90%) to the mammalian counterparts. Functional expression of CREB has confirmed that the newly cloned cDNA encodes a functional protein that can bind to canonical CRE and transactivate gene promoter with tandem repeats of CRE sites. In primary cultures of grass carp pituitary cells, SRIF treatment dose-dependently suppressed cAMP production and GH mRNA level with a concurrent rise in "steady-state" CREB mRNA expression. This increase in CREB mRNA levels could be partly attributed to the enhancement of transcript stability for CREB mRNA after SRIF treatment. The stimulatory effect of SRIF on CREB mRNA expression was mimicked by the AC inhibitor MDL12330A and PKA inhibitor H89. The AC activator forskolin or the cAMP analog CPT-cAMP, in contrast, was effective in reducing the basal expression of CREB mRNA in carp pituitary cells. In parallel experiments with GH3 cells transfected with a reporter construct carrying the 5' promoter of grass carp GH gene, over-expression of carp CREB was found to be inhibitory to basal GH promoter activity. Using 5' deletion analysis, the CREB responsive sequence could be mapped to the region between -742 to -646 of the grass carp GH promoter. These results, as a whole, suggest that SRIF, by inhibiting the AC/cAMP/PKA pathway, can up-regulate CREB gene expression at the pituitary level to inhibit GH gene expression in fish models.

FUNCTIONAL ROLE OF ACTIVIN AS A PARACRINE REGULATOR OF GROWTH HORMONE GENE EXPRESSION IN GRASS CARP PITUITARY CELLS

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Previous studies have shown that activin can regulate growth hormone (GH) release in mammals. However, the role of activin in modulating GH synthesis at the pituitary level is largely unknown. In this study, molecular cloning of activin β B was conducted in Chinese grass carp and its functional role in regulating GH gene expression was examined *in vitro* in primary cultures of grass carp pituitary cells. The full-length cDNA of grass carp activin β B was isolated by nested PCR coupled to 5'/3' RACE. The open reading frame of the newly cloned cDNA contains the coding region of the signal peptide, pro-peptide, and mature peptide of activin β B. The mature peptide is highly homologous (>90%) to activin β B reported in other species. Protein modeling also revealed that the 3-D structure of activin β B is highly conserved between the fish and human. To confirm the functionality of the carp activin β B cDNA, function expression of activin β B was conducted in CHO cells and the conditioned media containing grass carp activin β B was effective in stimulating the promoter activity of a pAR3-Lux reporter construct through the Mix2 activin-responsive elements. In the grass carp, the transcripts for activin β B were found to be widely expressed in various tissues. At the pituitary level, activin β B mRNA was detected in gonadotrophs and lactotrophs but not in somatotrophs. Besides, treatment of grass carp pituitary cells with activin B resulted in a dose-dependent decrease in "steady-state" GH mRNA levels. In contrast, removal of endogenous activin using follistatin, a physiological activin-binding protein, increased GH mRNA expression in a dose-related manner. To test the effects of activin on GH transcript stability, GH mRNA clearance studies was performed in grass carp pituitary cells pretreated with the transcription inhibitor actinomycin D. In this case, treatment with activin B could enhance the clearance of GH mRNA with a drop in the half-life ($T_{1/2}$) of GH transcripts. By real-time PCR, activin B also reduced the levels of GH primary transcripts in carp pituitary cells, suggesting that GH gene transcription was suppressed during activin treatment. In parallel studies with GH₃ cells transfected with a luciferase reporter construct carrying the 5' promoter of grass carp GH gene, treatment with activin B was effective in reducing the promoter activity of GH gene and this effect could be mimicked by over-expression of SMAD2 and SMAD3, the downstream effectors of activin's actions. These results, as a whole, indicate that activin is expressed locally in the pituitary of grass carp and acts as a paracrine inhibitor of GH gene expression, presumably through stimulation of GH mRNA degradation and inhibition of GH gene transcription.

PREVALENCE OF OSTEOPOROSIS AND FRACTURES IN CHINESE PATIENTS CHRONICALLY TREATED WITH GLUCOCORTICOIDS

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Aims: A cross-sectional study was conducted to measure the bone mineral density and fracture prevalence in patients with autoimmune diseases treated with glucocorticoids in a regional hospital in Hong Kong.

Methods: 118 Chinese patients who have been put on chronic glucocorticoid therapy were asked to fill in an osteoporosis risk factors questionnaire and undergo a DEXA (Lunar) examination of lumbar spine and proximal femur to measure bone mineral density. Radiological examination of spine was done to detect any vertebral fractures. 82.2% (n=97) were female. 43% (n=42) were post-menopausal without estrogen replacement. Diagnoses included systemic lupus erythematosus (n=75), glomerulonephritis (n=22), rheumatoid arthritis (n=6), and others including mixed connective tissue disease, fibrosing alveolitis and dermatomyositis (n=15). The mean age was 42.4±11.8 years. The mean body weight and height were 56.0±9.8Kg and 157.5±7.3cm. The mean duration of glucocorticoid treatment was 111±81 months. Current daily dose of prednisolone was 9.0±6.1 mg/D and mean cumulative dose was 34.9±27.4g.

Results: The mean BMD of lumbar spine (L2-4) was 1.031±0.133 g/cm² at T-score of -0.85 SD and Z-score of -0.72 SD. The mean femoral neck BMD was 0.800 ±0.137 g/cm² at T-score of -0.91 and Z-score of -0.45. One-Sample T-test showed Z-score was statistically different from zero at the lumbar spine and femoral neck (p<0.001). 13.6% (n=16) of the patients had T-score less than -2.5 either at lumbar spine or at femoral neck. 11.9% (n=14) had history of fractures. 4.2% (n=5) had radiological evidence of vertebral fractures. 25% (9/42) of postmenopausal female on chronic glucocorticoid therapy and 3.6% (2/55) of premenopausal female experienced fractures. 14.3% (3/21) of male had fractures. Those who fractured had statistically significant lower T-score of -1.600 at lumbar spine compared to a mean T-score of -0.749 for those who didn't fracture.

Conclusions: Prevalence of osteoporosis and fractures were respectively 13.6% and 11.9% in patients on chronic steroid therapy. Post-menopausal female and male were at a higher risk for fracture. Those who have low lumbar spine BMD at T-score < -1.5 should be considered for prophylactic therapy against fractures.

GENETIC AND CLINICAL CHARACTERISTICS OF MATURITY-ONSET DIABETES OF THE YOUNG IN CHINESE PATIENTS

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Objective: To determine the prevalence of different types of maturity-onset diabetes of the young (MODY) in Chinese patients with clinical MODY, and to characterize their phenotypes.

Patients and methods: The study included 146 Clinical MODY families. The probands were screened for mutations in the hepatocyte nuclear factor (HNF)-1 α (MODY3), glucokinase (MODY2), and HNF-4 α (MODY1) genes. Antibody to glutamic acid decarboxylase (GAD-Ab) was measured in the probands with MODY of unknown cause (MODYX). Insulin resistance index as presented by the product of fasting plasma glucose and fasting C-peptide, and other clinical data were compared in sex, age and duration-matched MODY3 and MODYX patients.

Results: Thirteen families were found to have mutations in the HNF-1 α gene and two in the glucokinase gene. Four of the 12 different HNF-1 α mutations are newly identified novel mutations (Q243E, A311D, P379R and P488fsdelC). In 131 MODYX probands, 3% were found to be GAD-Ab positive and 60% were obese. Compared to matched MODY3 patients, MODYX patients had higher BMI and poorer glycemic control. They also had higher triglyceride, lower HDL and more hypertension. Fasting C-peptide and insulin resistance index were higher in MODYX group than MODY3 group.

Conclusion: MODY3 and MODY2 account for 9% and 1% respectively, of Chinese MODY. A majority of the MODY patients are due to defects in unknown genes and seem to be characterized by insulin resistance. Identifying new MODY genes may lead to better understanding of the pathogenesis of obesity, insulin resistance and common T2DM.

COMBINED ORBITAL IRRADIATION WITH SYSTEMIC STEROID IN COMPARISON WITH SYSTEMIC STEROID ALONE IN THE MANAGEMENT OF SEVERE GRAVES' OPHTHALMOPATHY

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Background: Systemic steroid therapy and orbital irradiation, either alone or in combination, have been used in the management of moderate to severe Graves' ophthalmopathy. To date, there is limited data regarding the efficacy and tolerability of combined steroid therapy and orbital irradiation. We therefore conduct a randomized controlled trial to address the efficacy, effectiveness and safety of this combination therapy.

Methods: In a single-blind randomized prospective study, 16 patients with moderate to severe active Graves' ophthalmopathy were randomly assigned to therapy with either steroid alone (Group A) or steroid combined with orbital radiotherapy (Group B). Outcome measures included NOSPECS scoring system, total eye score (TES), subjective eye score and extraocular muscle thickness as determined by either CT or MRI scans.

Results: 15 out of 16 patients completed the 1-year study. Group B had more reduction in TES than Group A at week 16, 24 and 52 [P=0.029, P= 0.006 and P=0.029 respectively]. TES declined more quickly in Group B than Group A. Patients in Group B had more improvement in soft tissue swelling, diplopia, visual acuity and intraocular pressure than those in Group A at week 52. There was no improvement in proptosis in both groups. Maximum thickness of the extraocular muscles was significantly reduced in Group B over 24 weeks. The subjective eye score was similar in both groups at 52 weeks.

Conclusion: Combined steroid therapy and orbital irradiation is more effective than steroid alone in the treatment of moderate to severe active Graves' ophthalmopathy. Differences between the 2 groups were observed at as early as 16 weeks, and persisted till 52 weeks.

THE EFFECT OF DIET AND ORLISTAT ON BODY WEIGHT AND LIPID PROFILES IN CHINESE PATIENTS WITH CORONARY ARTERY DISEASE (CAD), OBESITY AND AVERAGE TO MODERATE HYPERCHOLESTEROLEMIA

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Introduction: Orlistat is effective in reducing weight and improving lipid profiles in patients with obesity. Both obesity and hypercholesterolemia are common cardiovascular risk factors present in patients with CAD. Thus we would like to know the effect of diet and orlistat on weight and lipid profiles in local obese Chinese patients with known CAD.

Method: 30 Chinese patients with known CAD, BMI ≥ 25 kg/m² and LDLC ≥ 2.6 and < 4.1 mmol/L were enrolled from the medical clinic of a general hospital in Hong Kong. They were put on weight-reducing diet for 12 weeks. Those with BMI ≥ 25 kg/m² after dietary treatment received orlistat 120 mg thrice daily for 24 weeks. The body weight and lipid profiles before and after diet and orlistat treatment were compared using nonparametric statistical test.

Result: 30 patients were initially enrolled. 29 patients received treatment with orlistat. 1 patient subsequently defaulted and another patient withdrawn from the trial because of acute myocardial infarction.

	12 weeks of dietary treatment			
	Before	After	% change	P-value
BW (kg)	76.8 \pm 8.0	75.5 \pm 8.5	- 1.6	< 0.001
BMI (kg/m ²)	28.6 \pm 3.4	28.1 \pm 3.5	- 1.7	0.001
TC (mmol/L)	5.32 \pm 0.76	5.16 \pm 0.68	- 3.0	0.121
TG (mmol/L)	2.10 \pm 1.01	1.86 \pm 0.86	- 11.4	0.084
HDLc (mmol/L)	1.03 \pm 0.17	1.04 \pm 0.17	+ 1.0	0.990
LDLC (mmol/L)	3.33 \pm 0.62	3.27 \pm 0.50	- 1.8	0.405

	12 weeks of diet followed by 24 weeks of diet and Xenical			
	Before	After	% change	P-value
BW (kg)	76.8 \pm 8.2	73.3 \pm 9.2	- 4.6	< 0.001
BMI (kg/m ²)	28.6 \pm 3.6	27.3 \pm 3.7	- 4.5	< 0.001
TC (mmol/L)	5.30 \pm 0.78	4.89 \pm 0.69	- 7.7	0.001
TG (mmol/L)	2.14 \pm 1.06	1.93 \pm 1.03	- 9.8	0.254
HDLc (mmol/L)	1.01 \pm 0.17	1.02 \pm 0.18	+ 1.0	0.732
LDLC (mmol/L)	3.34 \pm 0.64	3.05 \pm 0.44	- 8.7	0.030

Conclusion: Diet alone significantly reduced body weight while diet with orlistat significantly reduced body weight and improved TC and LDLC in local obese Chinese patients with CAD and average to moderate hypercholesterolemia.

GLYCEMIC CONTROL AND ENDOTHELIAL FUNCTION IN CHINESE SUBJECTS WITH TYPE 1 DIABETES MELLITUS

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Insulin glargine is a long-acting recombinant human insulin analogue. It has a stable activity profile with no pronounced peaks, and enables intensifying glycemic control in type 1 diabetes mellitus (DM). As endothelial dysfunction in diabetic patients has been linked to poor glycemic control, we have determined whether intensive glycemic control is able to reverse endothelial dysfunction in type 1 DM.

Twenty-three Chinese subjects (52% male) with type 1 DM were recruited. Their baseline endothelium-dependent and independent vasodilations of the brachial artery measured by high resolution vascular ultrasound were compared with 20 age-matched healthy controls (50% male). The measurements were repeated in the diabetic patients 24 weeks after incorporating glargine to their basal bolus regimen.

The age of controls and diabetic patients were 42.0 ± 4.0 and 37.5 ± 11.2 years respectively. The diabetic patients (duration of DM: 14.5 ± 7.5 years) had impaired endothelium-dependent vasodilation compared to controls ($6.2 \pm 2.2\%$ vs 9.1 ± 3.5 respectively, $p < 0.01$), whereas endothelium-independent vasodilation was similar ($16.6 \pm 5.9\%$ vs 18.1 ± 6.4 respectively). After 24-weeks' treatment with glargine, there were significant improvement in glycosylated hemoglobin ($7.0 \pm 0.7\%$ vs 8.4 ± 0.8 at baseline, $p < 0.001$), total cholesterol (4.4 ± 0.8 mmol/L vs 4.9 ± 0.7 , $p < 0.01$), and LDL-cholesterol (2.4 ± 0.7 mmol/L vs 2.8 ± 0.6 , $p < 0.01$) levels. Plasma HDL-cholesterol, triglyceride levels, systolic and diastolic blood pressure remained unchanged. Their body weight increased significantly (60.0 ± 9.0 kg vs 58.2 ± 9.3 at baseline, $p < 0.01$). There was no significant change in both endothelium-dependent ($5.8 \pm 3.6\%$ vs 6.2 ± 2.2 at baseline) and independent ($14.0 \pm 5.6\%$ vs 16.6 ± 5.9) vasodilation.

We conclude that Chinese subjects with type 1 DM have impaired endothelium-dependent vasodilation. Insulin glargine therapy can intensify glycemic control and improve some of the cardiovascular risk factors, but these effects do not translate into improvement in endothelial function.

PLASMA PHOSPHOLIPID TRANSFER PROTEIN ACTIVITY AND SUBCLINICAL INFLAMMATION IN TYPE 2 DIABETES MELLITUS

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Phospholipid transfer protein (PLTP) transfers phospholipids between lipoproteins, and plays an essential role in HDL metabolism. The regulation of PLTP is poorly understood and recent evidence suggests that PLTP activity increases during acute phase response. Since type 2 diabetes is associated with chronic subclinical inflammation, the objective is to determine whether inflammation modulates PLTP activity in diabetes. Plasma PLTP activity was assayed by measuring the transfer of radiolabelled phosphatidylcholine from liposomes to HDL and high sensitivity C-reactive protein (CRP) by immunoturbidimetric assay in 280 type 2 diabetic patients and 105 controls. Plasma PLTP activity (2364 ± 651 nmol/ml/h vs 1880 ± 586 in control, mean \pm SD, $p < 0.01$) and CRP [1.64 (0.89 – 3.23) mg/l vs 0.99 (0.53 – 2.33), median (interquartile range), $p < 0.01$] were increased in diabetic subjects. PLTP activity correlated significantly with age, BMI, HbA1c, log(CRP) and apolipoprotein AI and B in diabetic subjects. General linear model analysis showed that only apolipoprotein AI, age, BMI and log(CRP) were independent determinants of PLTP activity. In conclusion, PLTP activity is increased in diabetes and apolipoprotein AI is a major determinant of PLTP activity. There is also an independent association between CRP and PLTP activity, suggesting that subclinical inflammation may influence PLTP activity in diabetes.

REPRODUCIBILITY OF BEDSIDE CARDIOVASCULAR AUTONOMIC TEST IN NORMAL VOLUNTEERS AND TYPE 2 DIABETES

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Purpose of Study: Cardiovascular autonomic neuropathy (CAN) is common in both type 1 & 2 diabetic patients, and is associated with increased mortality. The clinical manifestations include impairment of exercise tolerance, orthostatic hypotension and silent ischemia or myocardial infarction. Early detection, preferably in the sub-clinical stage, by simple bedside CAN tests is important. It is not known how reproducible the tests are in local normal individuals and type 2 diabetic patients. The aim of the study is to assess the reproducibility of bedside CAN tests.

Methods: 16 healthy volunteers and 12 type 2 diabetic patients were recruited. Bedside CAN tests were performed twice for each subject within one week. No subject had signs of heart failure, arrhythmias or severe proliferative diabetic retinopathy. None were taking any medication that might affect the results during the test. Bedside CAN tests included: heart rate response to standing up, deep breathing and Valsalva maneuver and blood pressure response to standing up and sustained handgrip. The Ewing's criteria were used to classify the results (normal, early, definite, severe and atypical).

Summary of Results: For normal volunteers, 16 completed the study. 8 of them were males, 8 females. Their age ranged from 22 to 55 (mean 31) years. For type 2 diabetic patients, 5 were males, 7 females. Their age ranged from 42 to 70 (mean 58) years. By using the Ewing's criteria, all normal volunteers had normal results except for one atypical result in the first set of test (abnormal blood pressure response to sustained handgrip). In diabetic patients, the results were: 2 normal, 5 early, 1 definite and 4 severe CAN. None of them required re-classification in the second set of tests. 2 patients failed to perform Valsalva maneuver and one patient failed to perform sustained handgrip in both sets of tests.

Conclusions: Good reproducibility of bedside CAN tests was demonstrated in both normal individuals and type 2 diabetic patients. In no volunteer or patient did a repeat bedside CAN tests lead to re-classification of status by Ewing's criteria. Little extra information is gained by repeating the test.

THE ROLE OF TESTOSTERONE IN THE DETERMINATION OF SEXUAL DIMORPHISM OF ADIPONECTIN

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The fat-derived hormone adiponectin circulates in blood as several oligomeric complexes, and each of them might have distinct functions. However, the mechanisms that regulate the production of adiponectin oligomeric complexes are poorly understood. Here we demonstrated that the sex hormone testosterone might play a role. Gel filtration analysis revealed that the circulating adiponectin existed as the forms of high molecular weight (HMW, ~660kDa), middle molecular weight (MMW, ~440 kDa) and low molecular weight (LMW) in both human and mice. The concentration of the HMW form of adiponectin in female was significantly higher than that in male, while there were no gender differences for the other two forms. . Castration induced a dramatic elevation of the HMW form, but had no effect on either the MMW or the LMW form in male mice. Testosterone treatment of both sham-operated and castrated mice caused a specific reduction of the HMW form of adiponectin in the circulation, while neither MMW nor LMW was affected. Ex vivo studies on primary rat adipocytes revealed that testosterone selectively impeded the secretion of the HMW form of adiponectin, while it had no effect on its intracellular assembly. The inhibitory effect of testosterone on secretion of HMW form of adiponectin was largely restored by the transcription inhibitor actinomycin D. The selective inhibition of the HMW form of adiponectin by testosterone might contribute to the sex dimorphism of adiponectin, and could partly explain why men have higher risk to insulin resistance and atherosclerosis.

THE USE OF ALDOSTERONE-RENIN RATIO (ARR) AS A DIAGNOSTIC TEST FOR PRIMARY HYPERALDOSTERONISM AND ITS TEST CHARACTERISTICS UNDER DIFFERENT CONDITIONS OF BLOOD SAMPLING

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Aims: Recent reviews recommended the use of the aldosterone/renin ratio (ARR) to screen for primary hyperaldosteronism. However, widely different cutoff levels have been proposed and test characteristics of ARR under different conditions of sampling are not known. This study aims to 1. Assess the usefulness of ARR and identify an optimal ARR cutoff value in the Chinese population; and 2. Study the effect of conditions of blood sampling on the performance characteristics of ARR as a screening test in primary hyperaldosteronism.

Methods: We conducted a retrospective review among 45 subjects with carefully validated diagnoses of primary hyperaldosteronism and 17 subjects with essential hypertension to study the utility of ARR. 62 patients with 75 sets of plasma renin activity (PRA), aldosterone and ARR values from postural study and 48 sets of values from saline suppression test were analyzed. 94% of these subjects underwent investigations because of hypokalemic hypertension.

Results: ARR yielded larger areas under curve in receiver operating characteristics curve than PRA or aldosterone under all conditions of testing. ARR cutoff levels were significantly affected by the condition of testing (table 1). Depending on posture and time of day, it varied from 363 to 970 pmol/L per $\mu\text{g/L.h}$ in our study population. Blood sampling in the morning after 30 minutes in the seated position, using a cutoff value of 654 pmol/L per $\mu\text{g/L.h}$ (23.6 ng/dL per ng/mL.h), can achieve a sensitivity and specificity of 96.8 (83.2-99.5)% and 94.1 (71.2-99.0)% respectively.

Conclusions: When using ARR for screening primary hyperaldosteronism, posture and time of sampling should be standardized both within and between centres to minimize the variability in cutoff levels that currently exists. For screening a population of hypertensive outpatients, it would probably be most convenient to sample blood in the morning after 30 minutes in the seated position.

Parameter evaluated	Condition of blood sampling	Cutoff value with highest accuracy	Sensitivity (95% CI)	Specificity (95% CI)	LR+	LR-
ARR	0900h recumbent	970	96.5 (87.9-99.5)	100.0 (81.3-100.0)	∞	0.04
ARR	1300h ambulatory	363	96.5 (87.9-99.5)	88.9 (65.2-98.3)	8.68	0.04
ARR	Pre-saline (morning seated)	654	96.8 (83.2-99.5)	94.1 (71.2-99.0)	16.45	0.03
ARR	Post-saline	512	100.0 (88.7-100.0)	100.0 (80.3-100.0)	∞	0.00

Table 1. Cutoff values corresponding to the highest accuracy (minimal false positive and false negative results) and sensitivity, specificity, positive (LR+) and negative (LR-) likelihood ratios at these threshold values for aldosterone-renin ratio (ARR), aldosterone and plasma renin activity (PRA) under different test conditions. Units for ARR, aldosterone and PRA are pmol/L per $\mu\text{g/L.h}$, pmol/L, and $\mu\text{g/L.h}$ respectively.

AN EXTENDED FOLLOW-UP STUDY OF ADRENOCORTICAL FUNCTION OF CONVALESCENT HEALTH-CARE WORKERS WHO HAD RECEIVED HIGH DOSE STEROIDS FOR THE TREATMENT OF SEVERE ACUTE RESPIRATORY SYNDROME (SARS)

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Background: Convalescent SARS patients still suffered from long-term consequences and morbidity. We previously reported prolonged adrenal suppression in a group of health-care workers who had received high dose steroids for SARS as evaluated by low dose adrenocorticotropin (ACTH) test. We now report their follow-up assessment at 1 year.

Method: We prospectively evaluated adrenal reserve of 40 health-care workers with SARS by low-dose (1 μ g) ACTH test at 8 and 14 weeks after cessation of steroids. The tests were repeated at 26th and 52nd weeks. Insulin tolerance tests would be performed for confirmation if they agreed.

Results: By using the 30-minute serum cortisol concentration of at least 550 nmol/l as the cut-off point for adequate adrenal response, 37.5% (15/40) and 35% (14/40) patients had impaired adrenal functions by 26th week and 52nd week respectively. After correction for 3 defaulters, at least 27.5% (11/40) patients still had subnormal cortisol response. Their mean 30-minute cortisol concentration was 461 (range 341-549) nmol/l. Insulin tolerance tests were performed in 4/11 patients, all of them had peak serum cortisol less than 550 (range 462-525) nmol/l confirming persistent adrenal suppression. Their anterior pituitary functions were within normal limits. The prevailing symptoms did not correlate with the degree of adrenocortical function.

Conclusions: This is the first reported long-term follow-up evaluation of endocrine functions of post-SARS patients. Prolonged adrenal suppression, mostly of mild degree (30-minute serum cortisol > 400nmol/l), was observed in a considerable proportion of convalescent SARS patients.

ENDOCRINOLOGICAL OUTCOMES IN PATIENTS WITH CRANIOPHARYNGIOMAS IN HONG KONG

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Background: Craniopharyngioma is an uncommon benign intracranial tumour in adult population. Most tumours appear as suprasellar masses. Adult patients usually present with endocrine dysfunctions. Common endocrine symptoms include decreased libido, erectile dysfunction and secondary amenorrhoea. Surgery is usually the preferred primary treatment. In the management of tumour recurrence and residual lesions, radiotherapy and other new modalities of treatment are considered. Hormonal deficits are common after surgical treatment and radiotherapy.

Materials and methods: In a retrospective study carried out by reviewing the medical records of all patients with craniopharyngiomas followed up in the pituitary clinic between 1993-2003, a total of 27 patients were evaluated (13 M, 14 F). Age at the time of diagnosis ranged from 14-72 with median age 44.

Results: At presentation, hypogonadism was found in 75% of patients. Secondary adrenal failure and secondary hypothyroidism were found in 55% and 60% of patients, respectively. Diabetes insipidus was evident in 15% of the patients. 40% of the patients at initial presentation had hyperprolactinaemia. After the treatments were given, hypocortisolism and hypothyroidism were observed in 78.3 % and 65.2% of the patients, respectively. Hypogonadism occurred in 73.9 % of cases. Diabetes insipidus was evident in 73.9% of the cases.

Conclusion: In this series, the rate of hypocortisolism and hypothyroidism at presentation were higher than those reported in the literature [1]. In addition, the patients in this series had higher incidence of hypocortisolism, hypothyroidism and diabetes insipidus after receiving the treatment. We postulated that late presentation and aggressive treatment strategy might explain these findings.

Reference

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CALMODULIN AS A NOVEL MEDIATOR FOR FEEDBACK CONTROL OF GROWTH HORMONE GENE EXPRESSION BY INSULIN-LIKE GROWTH FACTOR IN GRASS CARP PITUITARY CELLS

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Calmodulin (CaM), the Ca^{2+} sensor in eukaryotic cells, is essential for biological functions mediated through Ca^{2+} -dependent mechanisms. Modulation of CaM gene expression at the pituitary level, however, has not been fully characterized. In this study, the functional role of CaM in the feedback control of growth hormone (GH) gene expression by insulin-like growth factor (IGF) was examined in grass carp pituitary cells. To establish the structural identity of CaM expressed in the grass carp, two CaM cDNAs, CaM-S and CaM-L, were isolated from the carp pituitary using nested PCR coupled to 3'/5' RACE. CaM-S was found to be a truncated form of CaM-L, and the ORFs of these cDNAs encode a 149 a.a. protein sharing the same primary structure with CaMs reported in mammals, birds, and amphibians. These cDNAs are phylogenetically related to the CaM I gene subfamily and their transcripts are ubiquitously expressed in the grass carp. In primary cultures of grass carp pituitary cells, IGF-I and -II were effective in inducing CaM mRNA expression with a concurrent drop in GH mRNA levels. These stimulatory effects on CaM gene expression were not mimicked by insulin and appeared to be a direct consequence of IGF activation of CaM gene transcription without altering CaM transcript stability. IGF-induced CaM mRNA expression in grass carp pituitary cells could be blocked by the inhibitors for PLC, PKC, and PI3K. The inhibitors for $\text{P}_{42/44}^{\text{MAPK}}$, $\text{P}_{38}^{\text{MAPK}}$, and JNK were not effective in this respect. In parallel studies, CaM antagonism also blocked the inhibitory effects of IGF-I and -II on GH mRNA expression whereas CaM over-expression markedly suppressed GH promoter activity. These results, as a whole, indicate that IGF feedback on GH gene expression is mediated by up-regulation of CaM gene expression at the pituitary cell level, presumably via activation of the PLC/PKC- and PI3K-dependent cascades.

DOPAMINERGIC REGULATION OF GROWTH HORMONE GENE EXPRESSION IN CHINESE GRASS CARP VIA PITUITARY D₁ RECEPTORS COUPLED TO THE cAMP/PKA-, PI3K- AND MAPK-DEPENDENT CASCADES

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Previous studies have shown that dopamine stimulates growth hormone (GH) release from grass carp pituitary cells through activation of pituitary D₁ receptors. However, it is still unclear if D₁ receptors are expressed in carp somatotrophs and the post-receptor signaling mechanisms for D₁ modulation of GH synthesis have not been examined. In this study, we have cloned the grass carp D₁ receptor by nested PCR coupled to 5'/3' RACE. The full-length cDNA obtained reveals that the a.a. sequence of grass carp D₁ receptor (363 a.a.) is highly homologous (60-94%) to the D₁ receptors reported in other species. Functional expression of the newly cloned cDNA in GH3 cells confirmed that grass carp D₁ receptors bound with the D₁ fluorescence probe tetramethylrhodamine-SCH23390 and this specific binding could be displaced by treatment with the D₁ agonist SKF77434 but not D₂ agonist Ly171555. Using RT-PCR coupled to laser capture microdissection, transcript expression of D₁ receptors was detected in isolated grass carp somatotrophs and lactotrophs but not in gonadotrophs. In primary cultures of grass carp pituitary cells, incubation with dopamine or the non-selective dopamine agonist apomorphine increased GH mRNA levels in a dose-dependent manner. These stimulatory effects were mimicked by the D₁ agonists SKF38393 and SKF77434 but not by the D₂ agonist Ly171555. Furthermore, SKF38393-induced GH mRNA expression could be blocked by the D₁ antagonist SCH23390 but not D₂ antagonist sulpiride. D₁ stimulation with SKF77434 also elevated cAMP production and levels of GH primary transcripts without affecting GH mRNA stability in grass carp pituitary cells. In parallel experiments, SKF77434-induced GH mRNA expression could be partially inhibited / abolished by the adenylate cyclase inhibitor MDL12330A, PKA inhibitor H89, P_{42/44}^{MAPK} inhibitors PD98059 and U0126, P₃₈^{MAPK} inhibitor SB203580 and PD169316, and PI3K inhibitors Wortmannin and Ly294002. The inhibitors for JNK, however, were not effective in this respect. These results, taken together, indicate that dopamine stimulates GH gene expression in grass carp pituitary cells by activating D₁ receptors functionally coupled to the cAMP/PKA-, MAPK-, and PI3K-dependent cascades. This dopaminergic stimulation is likely mediated via a direct action on the D₁ receptors expressed in carp somatotrophs.

HIGH LEVELS OF MET EXPRESSION IN THE CONTROL OF HEPATOCYTE GROWTH FACTOR-DEPENDENT INVASIVE GROWTH IN THE OVARIAN CANCER CELLS

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The hepatocyte growth factor (HGF) receptor, encoded by the *MET* oncogene, is over-expressed in 30% of ovarian cancer and plays a crucial role in controlling cell migration and tumor invasion. In this study, we investigated the molecular mechanisms by which HGF affect tumor aggressiveness in ovarian cancer using three human ovarian cancer cell lines: SKOV-3, CaOV-3 and OVCAR-3, which are representatives of common serous adenocarcinoma. We showed that HGF promoted cell scattering (dissociation) and invasion in SKOV-3 and CaOV-3, but not OVCAR-3. This difference was associated closely with a significantly higher level of Met expression in SKOV-3 and CaOV-3 than OVCAR-3. Reducing Met levels by siRNA or kinase activity by K252a abolished HGF-dependent cell scattering, indicating that high levels of Met are required for HGF-mediated cell migratory and invasion phenotype. Interestingly, Met expression was also enhanced by its ligand HGF, suggesting that HGF could further accentuate its effect through upregulation of Met. We next investigated signaling pathways critical for HGF-mediated cell motility and invasive phenotype using pharmacological inhibitors, and we demonstrated that both extracellular signal-regulated kinase (ERK) 1/2 and phosphatidylinositol 3-kinase (PI3K), but not protein kinase C (PKC) pathways were required for HGF-induced motility. Taken together, our data show that Met over-expression sensitizes ovarian cells to HGF stimulation, and the identification of downstream effectors may offer strategic targets for cancer therapy.

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PROTEOMIC ANALYSIS OF HUMAN OVARIAN SURFACE EPITHELIAL CELLS WITH GERMLINE MUTATIONS OF BRCA1

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BRCA1 has been implicated in the predisposition to early-onset breast and ovarian cancer. In this study, we report the first proteomic analysis to identify early and dynamic protein changes that may contribute to the malignant phenotype of ovarian cancers by comparing expression profiles of SV40 large T antigen-transfected, normal human ovarian surface epithelium (OSE), the tissue of origin of >90% ovarian cancer, from women carrying inherited mutations of BRCA1 with those from women without such mutations. Of >1500 protein spots displayed by two-dimensional gel electrophoresis, 18 were found to be differentially regulated after mutation. Proteins that displayed at least a 2-fold overexpression were chaperone proteins BiP, 94-kDa glucose-regulated protein, and α 2-microglobulin; annexin A2 and A11, key components of lipid trafficking; manganese-containing superoxide dismutase, an oxidative enzyme; and transgelin, expression of which regulates cytoskeletal architecture. Proteins that exhibited under-expression include glycolytic enzymes, heat shock protein 27 and tropomyosin. Some of the proteins identified are already known to be up- or down-regulated in ovarian cancer, and consistent with the previous identification of preneoplastic features in ovarian epithelial cells of high-risk individuals, but several are novel candidates. Many of the proteins identified are associated with protein synthesis or metabolism, which could potentially explain the observable differences in phenotypic or growth characteristics between OSE cells without or with BRCA1 mutation. Together, this study revealed differential expression patterns which may serve as molecular markers in early disease detection and provided insights into the molecular processes underlying the tumor suppressor function of BRCA1 in ovarian carcinogenesis.

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EXPRESSION PROFILING OF ENDOMETRIUM FROM WOMEN WITH NATURAL AND HYPERSTIMULATED CYCLE DURING IN VITRO FERTILIZATION TREATMENT

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Objective: To determine the expression profiles of endometria for patients with excessive ovarian response and to identify candidate genes as markers for hyperstimulation.

Method: Retrospective, controlled study and Affymetrix GeneChip™ analysis.

Setting: University infertility clinic and laboratory.

Method: Endometrial biopsies from four patients with serum estradiol concentration on the day of HCG > 20,000pmol/L (excessive responder), and 4 natural-cycle (natural) control patients were collected for total RNA isolation. The mRNA expression profiles were studied using Affymetrix UA-133 array, a high density oligonucleotide microarray comprising more than 22,000 genes. The results were analyzed using Cluster and TreeView programs (<http://rana.lbl.gov/EisenSoftware.htm>).

Result(s): About 50% (~11,000) genes were detected using the Affymetrix array for each sample. Cluster analysis demonstrated genes such as glycodelin, stanniocalcin, IGF2, complement component 4-binding protein α , cytokine receptor-like factor 1 were highly expressed (>2-fold) in all samples from the excessive responder. Interestingly, genes involve in ion transport such as transmembrane 4 superfamily member 4, solute carrier family 7, cystic fibrosis transmembrane conductance regulator (CFTR) were down-regulated (>2-fold) in all samples when compared to the natural cycle.

Conclusion(s): The present study identified known and new candidate markers that may unveil the underlying mechanisms on endometrial response and receptivity.

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UBIQUITINATION OF OCCLUDIN IN THE REGULATION OF TIGHT JUNCTION BARRIER IN THE TESTIS

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Throughout spermatogenesis, inter-Sertoli tight junctions (TJs) that constitute the blood-testis barrier must be disassembled and reassembled to permit the timely movement of preleptotene and leptotene spermatocytes from the basal to the adluminal compartment of the seminiferous epithelium. However, the mechanism and the participating molecules that regulate the bioavailability of TJ proteins are entirely unknown. Using Sertoli cell culture, it was shown that there was an increase in occludin level, concomitant with a reduction of an E3 ubiquitin ligase, Itch, at the time when inter-Sertoli TJs were assembled. By co-immunoprecipitation, occludin was shown to associate with Itch at the TJs. A novel interaction between Itch and UBC4, an ubiquitin-conjugating enzyme was identified. When TJs were disrupted by dibutyryl-cAMP (db-cAMP), an increase in protein levels of Itch and UBC4 along with a significant reduction in endogenous occludin was detected. These results seemingly suggest that the interaction of Itch and UBC4 on occludin is potentially involved in regulating Sertoli TJ dynamics. Addition of a proteasome inhibitor, MG-132, into Sertoli cells cultured with db-cAMP blocked the db-cAMP-induced occludin loss *in vitro*. Accumulations of ubiquitin-conjugated and Itch-conjugated occludin were detected in Sertoli cells cultured in the presence of both MG-132 and db-cAMP. These results suggest that MG-132 prevented db-cAMP-induced TJ disruption by altering the rate of occludin degradation. Taken collectively, the results reported herein support the notion that db-cAMP-induced TJ disruption was mediated by an induction of Itch protein expression, which in turn triggered the ubiquitination of occludin resulting in TJ disruption.

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VASCULAR ENDOTHELIAL GROWTH FACTOR AND VEGF-RELATED PEPTIDES IN HCG-INDUCED TESTICULAR ANGIOGENESIS

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A single subcutaneous (s.c.) injection of human chorionic gonadotrophin (hCG) (100 IU) induces significantly increases in endothelial cell proliferation and blood vessel density in the testes of adult rats, reaching peak levels at 48 and 72 hours post-hCG, respectively. Though this angiogenic response could be related to the presence of vascular endothelial cell growth factor (VEGF) in the testis, the constitutively high expression of this peptide in Leydig cells causes some concern, and also the involvement of other VEGF-related peptides (VEGF-B, -C and -D, and placental growth factor, PlGF) remains unclear. The aims of the present study were to examine the involvement of VEGF and VEGF-related peptides in mediating the hCG-induced endothelial cell proliferation in testes of normal adult rats and rats that had their Leydig cell function suppressed by exogenous testosterone treatment. Sprague-Dawley rats (400-450 g) were used. The expression of VEGF-related peptides was examined in the testis (and testicular cell lines) using RT-PCR, Western blot and immunohistochemistry. Suppression of Leydig cell function was achieved after 8-week treatment with 3 cm subcutaneous silastic implants containing testosterone. A single s.c. injection of 100 IU hCG (or saline for the control) was given 48 h before the animals were killed for the collection of testes. Data (after normalization) were compared using non-parametric statistical tests. Using RT-PCR and Western blot, VEGF-A_{188, 164, 144, 120}, VEGF-B_{186, 167}, VEGF-C, VEGF-D and PlGF were demonstrated in adult rat testes, and both Leydig cells and Sertoli cells appeared to represent the source of their mRNA and proteins. Following Leydig cell suppression, their mRNA levels remained little affected while VEGF-A₁₆₄ and VEGF-C peptide levels showed significant decreases. Following hCG injection, the stimulation of endothelial cell proliferation in normal and Leydig cell-suppressed testes was similar, and this was accompanied by significant increases in the levels of VEGF-A₁₆₄, VEGF-B₁₈₆ and VEGF-D. In conclusion, the present study demonstrates that specific isoforms of VEGF-A, -B and -D are increased after hCG stimulation, and thus they may play a role in mediating the hCG-induced increase in endothelial cell proliferation in the testis. However changes in their levels do not correlated well with the degree of endothelial cell proliferation, thus the additional involvement of other pro-angiogenic or antiangiogenic factors should not be discarded.

LEPTIN EXPRESSION IN EMBRYOS Sired BY MALE GOLDEN HAMSTERS WITH ALL ACCESSORY SEX GLANDS REMOVED

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Objective: Previous studies showed that embryos sired by golden hamsters with all accessory sex glands (ASG) removed exhibited anomalous development. Leptin, the *obese* gene product, plays an important role in embryonic development. In the present study, we aim to study leptin in embryos sired by males with all accessory sex glands removed.

Materials and methods: Leptin mRNA was detected in oocytes and preimplantational embryos sired by males with all accessory sex glands removed (TX) and controls (SH) with RT-PCR. Leptin protein in oocyte and preimplantation embryos was detected by fluorescence immunostaining with confocal method. Data are summarized in Tables 1 & 2.

Results: Leptin mRNA was present in the oocyte, 1-, 2-, 4-cell embryos and blastocyst both from the TX and SH group. Leptin immunofluorescence was concentrated at one point on the surface of the oocyte and was unevenly distributed in the blastomeres. The overall intensity of leptin was lower in embryos from TX group than control. No difference was found in CRL of fetus sired by TX and SH males. An increase in fetal to conceptus ratio from 9 to 11 *dpc* was due to a dramatic increase in fetal growth and leptin production in the fetus. During 11 *dpc* stage, the ratio of fetal to conceptus weight was significantly lower in TX group than control ($P<0.01$). Accordingly, fetal leptin content in TX group was also lower ($P<0.05$).

Table 1. Growth of embryos sired by hamsters with all ASG removed. (N= no. of litters)

	9 <i>dpc</i>			11 <i>dpc</i>		
	N	CRL mm	F/F+P	N	CRL mm	F/F+P
SH	5	4.2±0.1	0.14±0.01	5	10.9±0.1	0.54±0.01
TX	5	4.5±0.1	0.15±0.01	5	11.0±0.1	*0.52±0.01

* $P<0.01$, Student's t-test

Table 2. Leptin expression in embryos sired by hamsters with all ASG removed. (N= no. of litters)

	Total leptin (ng) in 9 <i>dpc</i> conceptus			Total leptin (ng) in 11 <i>dpc</i> conceptus		
	N	Fetus	Placenta	N	Fetus	Placenta
SH	5	0.107±0.028	0.154±0.022	5	0.067±0.007	0.057±0.004
TX	5	0.097±0.027	0.110±0.017	5	*0.051±0.004	0.054±0.003

* $P<0.05$, Student's t-test

Conclusions: Leptin was expressed by oocyte. The expression pattern was polarized in embryos. The removal of paternal accessory sex glands delayed leptin expression in the preimplantation stage. The main source of leptin production shifted from placenta to fetus from 9 to 11 *dpc*. Leptin expression in concepti sired by males with all ASG removed was lower on day 9*pc* but increased on day 11 which coincided with a catch-up growth.

PREVALENCE AND THE USE OF SY153 AS MARKER OF AZFD MICRODELETION IN CHINESE MEN

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Objective: To study the prevalence of the azoospermic factor-d (AZFd) deletion and the polymorphic rate of the sequence-tagged site sY153 in the Hong Kong Chinese men with severe male factor infertility.

Methods: The presence of two Y-linked STS (sY152, sY153) and BPY2 gene located within AZFd were determined by multiplex polymerase chain reaction in 273 infertile men (86 non-obstructive azoospermia, 148 severe oligospermia and 39 obstructive azoospermia). 62 fertile men were recruited as controls.

Result(s): Deletion of sY152 and BPY2 was found in 4.4% (12/273) of infertile patients (4 non-obstructive azoospermia, 8 severe oligospermia and none in obstructive azoospermia). Men with these deletions also had deletion of sY153 and AZFc. Fertile men and men with obstructive azoospermia did not have these deletions. With the use of a pair of newly designed primers, sY153 was found to be polymorphic with an incidence of 5.3% (17/323) in the studied population. The polymorphism occurred in both fertile and infertile men with similar frequency.

Conclusion(s): Our data do not support the existence of a distinct AZFd region in Hong Kong Chinese population. With the use of appropriate primers, sY153 can be used as a marker for Y-chromosome microdeletion screening.

A PRELIMINARY STUDY ON THE ROLE OF LEPTIN IN SIGNALING BETWEEN EARLY EMBRYOS AND OVIDUCT

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Background: Successful pregnancy requires communication between the embryo and the maternal body. Leptin could be one of the molecules mediating such communication. Mutation of leptin or its receptor causes infertility. However, the role of leptin in the regulation of early preimplantation embryo development is unclear.

Objective: To study the expression and role of leptin and its receptor in the oviduct and preimplantation embryos of mouse

Materials and Methods: (1) Immunohistochemical staining of leptin and its receptor in the oviduct and preimplantation embryos; (2) Determination of implantation rate after transfer of mouse embryos with or without anti-leptin antibody.

Results: Leptin immunoreactivity was detected in early embryos at all stages of development, but not in oviduct. Oviductal epithelium contained immunoreactivity of leptin receptor with intensity comparable among oviductal tissues from estrus, pregnant and pseudopregnant mice. Leptin receptor and its phosphorylated form were also present in preimplantation embryos at different developmental stages. Transfer of embryos together with anti-leptin antibody significantly decreased ($P < 0.0001$) the implantation rate from 66% (79/120) to 31% (43/138).

Conclusion(s): Leptin may participate in early embryo development through autocrine and paracrine pathways.

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PREVALENCE OF THE METABOLIC SYNDROME IN HONG KONG CHINESE ADOLESCENTS

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Background:

We are seeing an increasing trend of type 2 diabetes being diagnosed at an earlier age among the adult population in Hong Kong. It is purported that these individuals already harbour the metabolic abnormalities including glucose intolerance during their adolescent years. However there is no local data on the prevalence of metabolic syndrome in this young age group.

Aim:

To estimate the prevalence of the metabolic syndrome using the National Cholesterol Education Program, Adult Treatment Panel III (NCEP) definition, among Hong Kong Chinese adolescents.

Population:

960 girls and 1156 boys of Chinese origin aged 11-20 years in secondary school were examined.

Methods:

Data on anthropometric parameters and fasting blood samples were collected in the school setting. The NCEP definition for adult was used for classification of subjects with the metabolic syndrome except for obesity, which was defined by waist circumference $\geq 90^{\text{th}}$ percentile.

Results:

The overall crude prevalence of metabolic syndrome was 2.0% (2.5% in boys and 1.6% in girls). No significant difference in the prevalence of the metabolic syndrome between boys and girls was found. The prevalence was 10.9% for elevated triglyceride, 2.4% for low HDL-C, 11.3% for obesity, 0.3% for impaired fasting glucose and 20.2% for hypertension. Of these school children, 24.8% have one, 6.9%, 1.7% and 0.3% have two, three and four of the five abnormalities. .

Conclusion:

There is a high prevalence of hypertension among this adolescent population followed by obesity and dyslipidemia which are also common. These are worrying findings in our young and apparently healthy adolescents with strong public health implications, calling for more education in lifestyle modification.

AUTHOR INDEX

Au, CL	P-S7	Lee, KF	C2, P-C1, P-S5, P-S9, P-S10
Auersperg, N	P-S4	Lee, WM	S2, P-S6
Chan, FKW	C1, C3, P-C3, P-C5	Lee, YL	P-S10
Chan, JCN	P-C8	Leung, DYL	C3
Chan, KW	C2, C4, P-C4, P-C6	Leung, WS	C4
Chan, LF	P-S7	Liao, SB	P-S8
Chan, M	P-C8	Liu, Y	P-S5
Chan, MK	C3	Lo, KW	P-C1
Chan, V	C2	Lui, WY	S2, P-S6
Chan, YW	C4	Ng, CM	C1, C3, P-C3, P-C5
Cheung, WKL	P-S5	Ng, EHY	P-S5, P-S9
Chiu, JF	P-S4	Ng, YW	C1, C3, P-C3, P-C5
Choi, CH	C1, C3, P-C3, P-C5	O, WS	P-S8
Choi, KL	C3	Ozaki, R	P-C8
Chow, CC	P-C7	Pon, YL	S1
Chow, PH	P-S8	Qiao, Q	P-C8
Chow, WS	P-C1	Rui, H	P-C1
Chu, HK	C4	Shek, CC	P-C5
Chung, HS	P-S7	Shiu, SWM	P-C2
Chung, MK	P-S9	Siu, SC	C2, P-C7
Cockram, C	P-C7	Siu, SMM	P-C6
Dan, QH	C2	So, WY	P-C8
Ehlen, T	P-S4	Sze, KL	S2
Fu, G	S3, P-S1	Tam, S	C2, P-C2
Fung, LM	C2	Tan, KCB	P-C1, P-C2
Fung, RSK	S4	Tiu, SC	C1-3, P-C3, P-C5
Fung, YS	C4	Tong, CY	P-C8
He, QY	P-S4	Tsang, MW	C2
Ho, CS	P-C8	Tse, HF	P-C1
Ho, PC	P-S5, P-S9	Tsui, PT	C4
Hoo, RLC	P-C4	Tuomilehto, J	P-C8
Hung, HFV	C4, P-C6	Wang, X	P-S2
Huo, L	P-S1	Wat, NMS	C2
Keung, YK	P-S3	Wong, AOL	S3, S4, P-S1, P-S2
Ko, GTC	P-C8	Wong, AST	S1, P-S3, P-S4
Ko, WKW	S3, S4, P-S1, P-S2	Wong, E	P-S4
Kong, APS	C1, P-C5	Wong, GWK	P-C8
Kung, AWC	L1	Wong, NP	P-C7
Krabsov` , L	P-S9	Wong, Y	P-C2
Lam, CWK	P-C8	Wong, YMB	P-C6
Lam, EPH	L2	Xu, A	P-C4
Lam, KSL	C2, P-C1, P-C4	Xu, JY	C2
Lam, MCW	P-C4	Xu, JS	P-S10
Lau, EYL	P-S9	Yeung, JL	P-S7

Yeung, WSB	P-S9
Yuen, HKL	C3
Yuen, KT	C3
Zhang, JL	P-C4
Zhou, HY	P-S3
Zhou, Y	P-S4