WELCOME

Dear Colleagues,

We are delighted that you have joined us for the 17th International Congress on Hormonal Steroids and Hormones & Cancer, which is the first time that the meeting is being hosted on African soil. It is our privilege to host you in this beautiful part of the world that is the winelands of Stellenbosch.

It is our aim that ICHSHC 2018 will promote valuable interactions and discussions within the field of steroid hormones and hormone-dependent cancers. Please take the time to enjoy both the excellent scientific programme as well as several social functions which are aimed at facilitating discussion and bringing people together.

Finally, we would like to acknowledge the many corporate and institutional sponsors that have made it possible for us to host this prestigious event.

Welcome to Stellenbosch!

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on behalf of the local organising committee
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PROGRAMME

MONDAY, 26 NOVEMBER 2018
17:30 – 19:30  REGISTRATION AND WELCOME FUNCTION
19:30 – 20:30  INTERNATIONAL ICHSHC COMMITTEE MEETING

TUESDAY, 27 NOVEMBER 2018
Hormone dependent cancers
CHAIR: Karl Storbeck, Stellenbosch, South Africa
09:00 – 09:20  Welcome  Karl Storbeck, Stellenbosch, South Africa: (Conference chair)
               Fernand Labrie, Quebec, Canada: (Chair of the international ICHSHC committee)

10:20 – 10:50  COFFEE/TEA BREAK

CHAIR: Evan Simpson, Edinburgh, UK and Bernd Groner, Frankfurt, Germany
10:50 – 11:15  Oral 1  Theresa Hickey, Adelaide, Australia: Activation of the androgen receptor inhibits growth of normal breast tissue and estrogen receptor-driven breast cancers
11:15 – 11:40  Oral 2  Paul Chapple, London, UK: Oncometabolite induced primary cilia loss in pheochromocytoma promotes cellular proliferation
11:40 – 12:05  Oral 3  Wayne Tilley, Adelaide, Australia: The Forkhead Transcription Factor FOXA1 directs an oncogenic androgen receptor cistrome in estrogen receptor negative breast cancer cells
12:25 – 12:45  Oral 5  Renate Louw-du Toit, Stellenbosch, South Africa: Unravelling the role of signal transduction pathways in progestin-mediated effects on breast cancer

12:45 – 14:00  LUNCH

CHAIR: Theresa Hickey, Adelaide, Australia and Hironobu Sasano, Sendai, Japan
14:00 – 14:45  Plenary 1  Nima Sharifi, Cleveland, USA: Steroid metabolism in prostate cancer – from cellular phenotype to clinical consequences
14:45 – 15:10  Oral 6  Vanessa Hayes, Sydney, Australia: Androgen-regulated TMPRSS2-ERG fusions are rare in prostate tumors from African men, yet linked to early-onset low-grade disease
15:10 – 15:35  Oral 7  Tea Lanisnik Rizner, Ljubljana, Slovenia: Altered levels of ABC, OATP and OST transporters may contribute to E formation in endometrial cancer
15:35 – 15:55  Oral 8  Monique Barnard, Stellenbosch, South Africa: Moving beyond canonical androgens in castration resistant prostate cancer

15:55 – 18:30  POSTER SESSION

18:30 – 22:00  BRAAI (South African Barbeque)

WEDNESDAY, 28 NOVEMBER 2018
Steroidogenesis, steroid metabolism and action (I)
CHAIR: Gary Hammer, Ann Arbor, USA
09:00 – 09:45  Plenary 2  Wulf Ullan, Cleveland, USA: The intracrinological science and clinical application of intravaginal prasterone in management of vulvo-vaginal atrophy
09:45 – 10:10  Oral 9  Rita Bernhardt, Saarbrücken, Germany: Adrenal steroid hydroxylases with new functions
10:10 – 10:35  Oral 10  Fernand Labrie, Quebec, Canada: Increased efficacy with age of the intracrine transformation of dehydroepiandrosterone (DHEA) into androgens in peripheral tissues

10:35 – 11:05  COFFEE/TEA BREAK

CHAIR: Jonathan Mueller, Birmingham, UK
11:05 – 11:50  Plenary 3  Gary Hammer, Ann Arbor, USA: Enigma of the Adrenal SHH Progenitor
12:15 – 12:40  Oral 12  Riana Bornman, Pretoria, South Africa: Androgen receptor blockage induced by DDT disrupts the pituitary-gonadal axis and results in a functional testosterone deficiency and risk for metabolic disease

13:00  TOURS – Delegates who have signed up depart with packed lunch
THURSDAY, 29 NOVEMBER 2018

Nuclear receptor signalling
CHAIR: Donita Africander, Stellenbosch, South Africa

09:00 – 09:45  Plenary 4
Sebastiaan Meijsing, Berlin, Germany: Binding ≠ function: Linking glucocorticoid receptor binding to the regulation of genes in the endogenous genomic context
Luc Furic, Melbourne, Australia: Translational regulation by ERα in hormone-dependent cancers
Ilma Simoni Brum, Porto Alegre, Brazil: Androgenic modulation of AR-Vs

10:35 – 11:00  COFFEE/TEA BREAK

09:45 – 10:10  Oral 13

10:10 – 10:35  Oral 14
Ilma Simoni Brum, Porto Alegre, Brazil: Androgenic modulation of AR-Vs

10:10 – 10:35  Orals 13 and 14

10:35 – 11:00  COFFEE/TEA BREAK

CHAIR: Wayne Tilley, Adelaide, Australia

11:00 – 11:25  Oral 15
Janet Hapgood, Cape Town, South Africa: Synthetic progestin contraceptives medroxyprogesterone acetate and norethisterone exhibit differential receptor-mediated effects on gene expression and HIV-1 replication

11:25 – 11:50  Oral 16
Wim Vanden Berghe, Antwerp, Belgium: Molecular biochemical characterization of selective glucocorticoid receptor activities of GSK866 analogues with cysteine reactive warheads

11:50 – 12:10  Oral 17
Jan Kroon, Leiden, Netherlands: Metabolic glucocorticoid receptor transcriptional activity is androgen-dependent

11:00 – 12:10  Orals 15 and 17

12:10 – 13:30  LUNCH

Steroidogenesis, steroid metabolism and action (II)
CHAIR: Nicolette Verhoog, Stellenbosch, South Africa

13:30 – 14:15  Plenary 5
Wiebke Arlt, Birmingham, UK: Androgens, PCOS and metabolic risk – dissecting the female andrometabolic syndrome

14:15 – 14:40  Oral 18
Louise Metherell, London, UK: Sphingosine-1-phosphate lyase (SGPL1) deficiency is associated with mitochondrial dysfunction

14:40 – 15:00  Oral 19
Janina Tokarz, Munich, Germany: 20alpha- and 20beta-reduction of glucocorticoids in humans: which role plays CBR1?

15:00 – 15:30  COFFEE/TEA BREAK

CHAIR: Jurek Adamski, Munich, Germany

15:30 – 15:50  Oral 20
Lina Schiffer, Birmingham, UK: Intracrine activation of 11-oxygenated androgens by AKR1C3 is increased by HSD11B1 inhibition

15:50 – 16:10  Oral 21
Desmaré Van Rooyen, Stellenbosch, South Africa: The metabolism of adrenal C11-oxy C21 steroids to C11-oxy androgens in the backdoor pathway

16:10 – 16:55  Plenary 6
William Rainey, Ann Arbor, USA: Adrenal aging, gene mutations and primary aldosteronism

16:55 – 17:05  Closing of the scientific programme
Amanda Swart, Stellenbosch, South Africa

19:00  CONFERENCE DINNER - buses depart STIAS at 18:30
Estrogen Receptor (ER) is the defining feature of luminal breast cancer. ER requires associated proteins to interact with the DNA, including FoxA1 and GATA3. GATA3 and FoxA1 are mutated in primary breast cancer and ESR1 (ER) is mutated in the metastatic context. We have explored the potential role of the GATA3 mutations, by engineering the common mutation into a cancer cell line and characterising the impact of these mutations on hormonal signaling. In parallel, we have conducted a global CRISPR screen to identify genes involved in endocrine response and have revealed a role for the BAF (Swi/Snf) complex in mediating treatment response. Unexpectedly, the response was the opposite for epigenetic inhibitors (BETi), suggesting that the BAF complex is required for ER antagonists to exert antiproliferative effects, but inhibits the response to BET inhibition. Given that the BAF complex is mutated in treatment resistant disease, we hypothesise that epigenetic inhibitors might show improved efficacy in BAF-mutant patients.

It is well established that most ER+ breast cancer also express Progesterone Receptor (PR) and Androgen Receptor (AR). Several labs, including ours, have recently implicated molecular cross-talk between PR/AR and the ER pathway, providing the opportunity for exploiting parallel hormonal pathways for therapeutic benefit. However, there are contradictory findings associated with the role of PR and progestogens in breast cancer and inappropriate extrapolations made from normal mammary gland biology to cancer contexts. To address this and to fully exploit the hormonal cross-talk, we have extended on our initial observations to characterise the nuclear receptor cross-talk in cancer models and primary tumour explant samples and to compare distinct PR ligands that have traditionally been linked with different physiological outcomes. In addition, we have initiated a clinical trial to explore the therapeutic potential of repurposing PR agonists for primary ER+ breast cancer.
Activation of the Androgen Receptor Inhibits Growth of Normal Breast Tissue and Estrogen-Driven Breast Cancers

Theresa Hickey, Luke Selth, Danial Roden, Esmaeil Ebrahimie, Heloisa Milioli, Suzan Stelloo, Wilbert Zwart, Carlo Palmieri, Jessica Finlay-Schultz, Carol Sartorius, Kee Ming Chia, Mun Hui, Alex Swarbrick, Elgene Lim, Jason Carroll, Wayne Tilley

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6. Cancer Research UK-Cambridge Institute, University of Cambridge, United Kingdom

The estrogen receptor alpha (ER) stimulates normal breast development but abnormal ER activity drives the majority of breast cancers. In contrast, the androgen receptor (AR) inhibits breast development and may restrain ER-mediated breast carcinogenesis. To investigate the genomic mechanisms involved, we interrogated single or dual hormone receptor activation in contemporary ex vivo and in vivo patient-derived models of normal and malignant breast tissue and a breast cancer cell line model (ZR75-1) that endogenously expresses ER and AR. Estrogen treatment induced a proliferative transcriptome in patient-derived explants (PDEs) of normal and malignant breast tissues cultured ex vivo while androgen treatment induced an anti-proliferative, anti-estrogenic transcriptome. The opposing effects of estrogen and androgen hormones on proliferative indices were also observed in ZR75-1 xenograft and patient-derived xenograft (PDX) models of ER-driven breast cancer. In these experiments, the action of natural androgen was mimicked by treatment with a clinically relevant selective AR modulator (SARM). Importantly, androgen or SARM treatment also inhibited in vivo growth of an ER-positive PDX model resistant to standard-of-care anti-estrogenic agents. ChIP-seq experiments revealed that activated AR reduced (4 hr in vitro; 5 days in vivo) estrogen-stimulated ER binding to chromatin, which curtailed ER-mediated transcription and resulted in long-term inhibition of tumour growth. Transcriptional profiling of androgen- or SARM-treated tumours revealed AR-associated gene signatures that were enriched in the least aggressive subtype of breast cancer (Luminal A) and were predictive of better disease outcomes. Altogether, our work suggests that AR-mediated genomic activity inhibits growth of normal breast tissues and can be induced to inhibit ER-driven malignant breast tissues, including those that are resistant to drugs currently used to treat this subtype of disease. The data supports the use of SARMs as a therapeutic means of preventing or treating ER-driven breast cancer.
Oncometabolite induced primary cilia loss in pheochromocytoma promotes cellular proliferation

Samuel M. O’Toole, David S. Watson, Lisa E.L. Romano, Michael R Barnes, Umasuthan Srirangalingam, William M. Drake, J. Paul Chapple

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University College London Hospital, London, United Kingdom

Primary cilia are sensory organelles involved in regulation of cellular signalling and cell division. Cilia loss is frequently observed in tumors, yet the responsible mechanisms and consequences for tumorigenesis remain unclear. We demonstrate that cilia structure and function is disrupted in human pheochromocytomas - endocrine tumours of the adrenal medulla. This is concomitant with transcriptional changes within cilia mediated signalling pathways, that are associated with tumorigenesis generally and pheochromocytomas specifically. Importantly, cilia loss was most dramatic in patients with germline mutations in the pseudohypoxia-linked genes SDHx and VHL. Using a pheochromocytoma-derived cell line, we show that hypoxia and oncometabolite induced pseudohypoxia are key drivers of cilia loss and identify that this is dependent on activation of an AuroraA/HDAC6 cilia resorption pathway. We also show cilia loss drives dramatic transcriptional changes associated with proliferation and tumorigenesis. Our data provide evidence for primary cilia dysfunction contributing to pathogenesis of pheochromocytoma by a hypoxic/pseudohypoxic mechanism and implicates oncometabolites as ciliary regulators. This is important as pheochromocytomas can cause mortality by mechanisms including catecholamine production and malignant transformation, while hypoxia is a general feature of solid tumours. Moreover, pseudohypoxia induced cilia resorption can be pharmacologically inhibited, suggesting potential for therapeutic intervention.
The Forkhead Transcription Factor FOXA1 Directs an Oncogenic Androgen Receptor Cistrome in Estrogen Receptor Negative Breast Cancer Cells

Wayne Desmond Tilley¹, Iza Denis¹, Luke Selth¹, Jessica L L Robinson¹, Hisham Mohammed¹, Jason S Carroll¹, Theresa E Hickey¹

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²Department of Oncology, University of Cambridge, Cambridge, UK

The estrogen receptor (ER) drives the majority (>70%) of breast cancers, a feature that requires the forkhead transcription factor FoxA1 to open chromatin for ER to bind DNA. In breast cancers that lack ER, the androgen receptor (AR) has been implicated as an oncogenic driver. In support of this concept, we have previously shown that AR genomically phenocopied ER in associating with FOXA1 and inducing a luminal gene expression profile in ER-negative breast cancer cells¹. Herein, we investigated the consequences of FoxA1 loss on AR signalling in this context. Transient knock-down of FoxA1 in the MDA-MB-453 cell line model inhibited cell proliferation but did not prevent AR from binding to DNA. Rather, the AR cistrome was substantially reprogrammed, with gain of AR binding at a large number of genomic loci. Genome-wide analysis of histone marks associated with active transcription (H3K4me1, H3K27Ac) demonstrated that the “AR reprogrammed” sites were pre-marked before the loss of FoxA1. Quantitative proteomic comparison of the chromatin-bound AR transcriptional complex in the absence of FoxA1 revealed an increased interaction with AP2α, a transcription factor associated with the AR cistrome in mouse epididymis. Stimulation of AR/AP2α interactions by FoxA1 knockdown was validated using proximity ligation assays in the MDA-MB-453 and MFM-223 cell line models. Transient knockdown of AP2α revealed this factor was required for AR to bind to loci associated with FOXA1 loss. Importantly, these AP2α-dependent/FoxA1-independent AR binding sites were associated with genes up-regulated in ER-positive (luminal) compared to more aggressive ER-negative (basal/mesenchymal) breast cancers. Overall, our findings suggest that loss of FoxA1 can result in a switch to AP2α-directed AR signalling, which is associated with reduced proliferative capacity and a more luminal phenotype in ER-negative breast cancers. These findings suggest the oncogenic activity of AR in this disease context may be dependent upon interaction with FOXA1.
Emerging roles of cholesterol in human breast cancer: Possible involvement of its intratumoral metabolism on its clinical behavior.

Hironobu Sasano¹, Shinkichi Kosaka², Hiroyuki Ueno³, Minoru Miyashita³, Takanori Ishida³, Ju-Yeon Moon³, Man-Ho Choi³, Keely M McNamara³
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One of the pivotal risk factors of breast cancer development is obesity. In addition, the use of statins has been reported to decrease the risk of breast cancer development and therefore cholesterol metabolism and action in breast cancer tissues could be interesting area of research. However, comprehensive study of intratumoral cholesterol pathways with comparison between matched normal tissues and/or among breast cancer subtypes and correlation with clinicopathological findings including clinical outcome have not been reported. Therefore, we first used GC-MS/MS analysis to quantify the levels of cholesterol as well as various precursors and metabolites (cholesterol esters, oxysterols and others) in 60 cases of breast cancer including different subtypes retrieved from the tissue bank at Tohoku University Department of Pathology. Among these cases, histologically normal breast tissues were available for comparison. Cholesterol esters was significantly higher in tumor than paired normal breast (p<0.05), suggesting increased cholesterol storage in cancer tissue. Among different subtypes, significant increases in 24-OHC and 27-OHC (p>0.05 for both) with a trend towards increased 7α-OHC were noted in the triple negative subtype. However, only 24-OHC was significantly different between normal and paired cancer samples with consistently higher levels observed in the normal tissues. In two way clustering analysis, four major clusters were detected, different from ER/PR/HER2 derived classification. We then studied immunohistochemistry (IHC) and RT-PCR of cholesterol metabolizing enzymes including 27-OHC and 24-OHC and 7αOHC in normal and cancer tissues in 60/80 cases above. 7α-OHC status was significantly higher in cancer than normal tissues and intratumoral status of CYP7A1 and CYP27A1 was significantly correlated with smaller tumor size and longer disease free survival (DFS) of the patients. Cholesterol and related molecules harbor multiple different roles in biological behavior of the breast cancer patients. Increased cholesterol storage seems important in pathogenesis and signaling through oxysterols being important in driving the more aggressive subtypes of breast cancer. In addition, intratumoral CYP27A1//27-OHC and CYP7A1/7αOHC status was significantly associated with good clinical outcome of the patients examined.
Clinical studies have suggested that menopausal hormone therapy is associated with an increased risk of developing breast cancer. Interestingly, the risk is higher in women using estrogen-progestin combined therapies than in women using estrogen-only therapies. However, these studies examined only a few progestins such as medroxyprogesterone acetate (MPA), norethisterone acetate (NET-A), levonorgestrel (LNG) and nomegestrol acetate. Considering that a variety of progestins with distinct structures and functions are available, it is possible that not all progestins would be harmful in terms of breast cancer. In this study, we directly compared the effects of a number of progestins on tumour cell behaviours such as proliferation and migration of the human T47D breast cancer cell line. Moreover, as the ERK1/2 and JNK signal transduction pathways are known to play crucial roles in growth, survival and metastasis, we also examined the role of these pathways on the above-mentioned processes. Results showed that all the progestins, except the newer, structurally unique progestin, drospirenone (DRSP), increased proliferation, migration and anchorage-independent growth of the T47D breast cancer cells to the same extent. Interestingly, we showed that unlike the earlier generation progestins, MPA, NET-A and LNG which upregulated several genes that play a role in breast cancer cell growth, DRSP had no effect. Moreover, our study revealed that the effects of the older progestins on T47D cell growth and migration are blocked in the presence of the highly selective ERK1/2 and JNK signal transduction pathway inhibitors. These results suggest that activation of the ERK1/2 and JNK pathways may be a mechanism by which the earlier generation progestins increase breast cancer risk.
Prostate cancer initiation and progression are reliant androgen stimulation of the androgen receptor (AR). Androgen deprivation therapy (ADT) by way of medical or surgical castration has been a mainstay of upfront therapy for advanced prostate cancer for over 7 decades. Eventually, castration-resistant prostate cancer (CRPC) occurs and subsequent therapies include agents that further block the androgen pathway with drugs such as abiraterone, which blocks extragonadal androgen synthesis, and enzalutamide, which directly antagonizes AR. Resistance to all of these treatment modalities involves inherited or acquired aberrations in steroid metabolism. The rate-limiting step of potent androgen synthesis from extragonadal precursor steroids is genetically regulated by a common germline variant in \( HSD3B1 \) (1245C) that encodes for 3β-hydroxysteroid dehydrogenase-1 (3β-HSD1). The \( HSD3B1 \) (1245C) variant encodes for a more stable enzyme that is present in approximately 1 in 2 individuals, enables dihydrotestosterone synthesis from extragonadal precursors, is a predictive biomarker of ADT-resistance and a predictive biomarker of sensitivity to extragonadal androgen synthesis inhibition. Further, the same enzyme and variant also regulate metabolism of abiraterone, a steroidal drug, to multiple biochemically active steroidal abiraterone metabolites that impinge on the AR axis. Resistance to enzalutamide is mediated by a switch from AR to glucocorticoid receptor (GR) and a concurrent intratumoral metabolic aberration that blocks cortisol inactivation, allowing accumulated cortisol to stimulate GR and tumor progression. Clinical implications of these studies will be discussed.
Androgen-regulated TMPRSS2-ERG Fusions Are Rare in Prostate Tumors from African Men, Yet Linked to Early-Onset Low-Grade Disease

Vanessa M. Hayes, James Blackburn, Riana Bornman

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Prostate cancer is developed through a series of specific genetic alterations. Transmembrane protease, serine 2 and v-ets erythroblastosis virus E26 homolog (TMPRSS2-ERG) gene fusions are the most common acquired alteration - resulting in androgen-dependent overexpression of ERG. Present in roughly 50% of all European-derived tumors, this fusion variant is notably halved in tumors from African Americans. Conversely, African-American men are at significant risk of presenting with high-risk disease. Limited studies have been performed within populations from the African continent. Here we determined the relationship between the presence of TMPRSS2-ERG fusion, as well as the observed racial disparity and clinical relevance, in a cohort of 181 Black South African men. At a frequency of less than 13%, we found the presence of the TMPRSS2-ERG fusion gene to be 92.62% predictive for a positive prostate cancer diagnosis ($P = 0.0031$), with a significant link to low-grade disease in younger patients ($P = 0.04656$). Arguably, the increase in TMPRSS2-ERG fusions may be directly related to increased androgen levels in younger men. Accordingly, we found the younger TMPRSS2-ERG fusion positive patients were more likely to present with higher expressing distal-junction-coordinated ERG isoforms. Our study suggests that the 2.1-fold increased risk for high-grade prostate cancer presentation in Black South Africans over African Americans may account for the almost 2-fold decrease in frequency of TMPRSS2-ERG fusion observed. Additional screening for non-TMPRSS2-ERG fusion gene alterations using a novel RNA CaptureSeq method, suggests that chromosomal translocations are unlikely events in prostate tumorigenesis in African patients.
Altered levels of ABC, OATP and OST transporters may contribute to enhanced E₂ formation in endometrial cancer

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Objectives: The aims of the study were: to examine capacity of endometrial cancer (EC) for formation of estradiol (E₂) from estrone-sulfate (E₁-S), to evaluate contribution of E₁-S transporters for E₂ formation and to assess the potential association between protein levels of E₁-S transporters, histopathological and clinical data. Methods: By qPCR we analyzed the expression of 20 SLCO, SLC and ABC genes encoding OATP, SOAT, OAT, OST and ABC transporters in model cell lines of EC, Ishikawa and HEC-1-A, and in paired samples of EC and adjacent control tissue. The levels of differentially expressed genes were evaluated by IHC. E₁-S metabolism studies were performed in tissue samples and model cell lines. Results: In model cell lines of EC the highest difference in expression was seen for SLCO1B3 and ABCG2 in HEC-1-A compared to control cell line HIEEC. This is in line with the enhanced E₁-S metabolism observed in HEC-1-A versus Ishikawa cells. In EC tissue we found increased E₂ formation via the sulfatase pathway and significant changes in expression of SLCO, SLC and ABC genes in postmenopausal patients. Genes ABCG2 and SLCS1B were 3.2-fold and 2.1-fold down-regulated, respectively, and ABCC1 was 1.5-fold up-regulated in EC versus adjacent control tissue. Also IHC revealed significantly lower protein levels of ABCG2 and SLCS1B (OSTβ) in EC versus control tissue. In patients without lymphovascular invasion gene SLCO1B3 was 15.6-fold up-regulated. Tumor grade had significant effects on expression of SLCS1B, with lower levels seen in high grade cancers. Conclusions: Our results suggest that ABC, OATP and OST transporters have important roles in E₂ formation in EC, where decreased levels of ABCG2 and OSTβ might be related to higher intracellular E₁-S concentrations and enhanced E₂ activation in EC tissue. More detailed investigation of individual transporters and their contribution to E₁-S uptake and efflux is currently in progress.
The intratumoral conversion of weak androgen precursors to more potent androgens fuels the development of castration resistant prostate cancer (CRPC). Traditionally only the canonical adrenal androgen precursors, dehydroepiandrosterone (DHEA) and androstenedione (A4), have been considered in this regard. These precursors are converted to the potent androgen 5α-dihydrotestosterone (DHT) by the well characterised 5α-androstanedione pathway. More recently we have provided evidence that the overlooked adrenal steroid 11β-hydroxyandrostenedione (11OHA4) is in fact the precursor to 11-ketotestosterone (11KT) and 11-keto-5α-dihydrotestosterone (11KDHT), which bind and activate the human androgen receptor (AR) with affinities and potencies similar to that of testosterone (T) and DHT, respectively. Moreover, we have previously demonstrated that 11KT and 11KDHT both induce AR-regulated gene expression and drive cell growth in androgen dependent prostate cancer cell lines. We have subsequently measured the circulating levels of adrenal derived 11-oxygenated androgens and their precursors in patients undergoing treatment for advanced prostate cancer and show that the precursor, 11OHA4, circulates at levels comparable to, or higher than, those of DHEA and A4, and that 11KT circulates at higher levels than T in castrated men. Furthermore, we have demonstrated that AKR1C3, the enzyme required for the activation of all androgen precursors, has an 8- to 22-fold catalytic preference for 11-oxygenated androgen substrates compared to the canonical substrates. In contrast, 17βHSD2 which catalyses the reverse inactivation reaction, demonstrates little to no substrate preference. We found that increased AKR1C3:17βHSD2 expression ratios, as measured in CRPC tissue, significantly favour the activation of 11-oxygenated androgens. Taken together with previous studies which found that 11KT and 11KDHT are metabolised at a significantly lower rate than T and DHT, we conclude that 11-oxygenated androgens may accumulate within CRPC tumours. This work highlights that the 11-oxygenated androgens may play an equally important role to canonical androgens in the development of CRPC.
Objective: To illustrate the marked difference between classical endocrinology with hormone distribution to all tissues, and the new science of intracrinology, through which each cell makes its own estrogens and androgens from the inactive precursor dehydroepiandrosterone (DHEA). These sex steroids are inactivated inside the same cells, with no influence in the other tissues, since only inactivated sex steroids are released in the blood. This mechanism avoids potential harmful effects related to an elevation of serum estradiol (E2).

Results/Observations: Evolutionary changes have resulted in about 30 steroid-forming tissue-specific enzymes being developed in peripheral tissue that transform DHEA, mainly of adrenal origin, into minute amounts of estrogens for a strictly local action. Humans, contrary to lower species, also possess intracellular steroid-inactivating enzymes, especially glucuronyl transferases and sulfotransferases, which prevent the release of a biologically significant amount of E2 in the circulation. DHEA being the unique source of sex steroids after menopause, serum E2 is thus maintained at low biologically inactive concentrations after menopause. DHEA secretion, unfortunately, starts decreasing from about age 30 years at different rates in different women. Moreover, there is no feedback mechanism to increase DHEA secretion when serum DHEA is low. It appears logical to replace DHEA locally in women suffering from vulvovaginal atrophy (VVA) (genitourinary syndrome of menopause, GSM). There is also developing evidence for an effect on hypoactive sexual desire disorder (HSDD) or inhibited sexual desire. Data obtained using a small dose of intravaginal DHEA confirm the mechanisms of intracrinology mentioned above which avoid biologically significant changes in serum E2. The low serum levels of estradiol, their precursors and metabolites were measured by liquid chromatography/tandem mass spectrometry assays validated according to the FDA guidelines. Conclusion: The symptoms and signs of VVA (GSM) can be successfully treated by the intravaginal administration of DHEA without safety concerns. There is also increasing evidence in support of a beneficial effect on female sexuality. The strategy exclusively replaces the missing vaginal cell-specific intracellular estrogens and androgens. This avoids systemic exposure and subsequent potential risks. In this way, the FDA black box warning can be eliminated.

Six cytochromes P450 are involved in steroid hormone biosynthesis. We investigated the role of steroid hormone intermediates and steroidal drugs on the function of these P450s. We found a direct stimulatory effect of estradiol and estrone onto pregnenolone formation in a reconstituted in vitro system with CYP11A1. Furthermore, we demonstrated the formation of new products by CYP11A1 from 11-deoxycorticosterone (DOC), androstenedione, testosterone and dehydroepiandrosterone (DHEA). Our results are the first evidence that sex hormones positively regulate the overall production of steroid hormones suggesting the need to reassess the role of CYP11A1 in steroid hormone biosynthesis. We also unraveled CYP21A2 to have a broader steroid substrate spectrum than assumed. We demonstrated that CYP21A2 is capable to metabolize DOC, RSS, androstenedione (A4) and testosterone (T). Moreover, the conversion of A4 rendered a product showing a hydroxylation at position C16-beta. The androgenic properties of this steroid metabolite, 16ßOHA4, were investigated and compared with A4. Both metabolites were shown to be weak agonists for the human androgen receptor. Moreover, the interaction of 16ßOHA4 with the aromatase (CYP19A1) was compared to that of A4, indicating that the C16 hydroxyl group does not influence the binding with CYP19A1. Interestingly, 16ßOHA4 was found to be present in a patient with 11-hydroxylase deficiency and in a patient with an endocrine tumor. Finally, we were able to demonstrate in-vitro that adrenal steroid hydroxylases are not only of striking importance for the biosynthesis of steroid hormones, but are also involved in the biotransformation of steroidal drugs such as anabolics and spironolactone leading to novel metabolites of their biotransformation. This is of high importance for understanding steroid hydroxylase function as well as for drug safety.
Increased efficacy with age of the intracrine transformation of dehydroepiandrosterone (DHEA) into androgens in peripheral tissues

Céline Martel¹, Fernand Labrie¹, Yuyong Ke¹, Jean-Nicolas Simard¹, Alain Bélanger²
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Objective: To estimate the efficacy of transformation of DHEA into androgens in peripheral tissues at different ages in women. Methods: DHEA, testosterone and a predominant androgen metabolite, namely androsterone glucuronide (ADT-G) have been measured in women aged 21-30 (n=31), 31-40 (n=88), 41-50 (n=34), 51-60 (n=58), 61-70 (n=47) and 71-79 (n=34) years using a validated LC-MS/MS methodology. Results: The average ratios of DHEA/ADT-G have been measured at 0.23 ± 0.02 (mean ± SEM), 0.15 ± 0.01, 0.20 ± 0.02, 0.16 ± 0.01, 0.13 ± 0.01 and 0.12 ± 0.01 in the different groups of women of increasing age mentioned above and ranging from 21 to 79 years of age. When looking at serum testosterone which reflects diffusion of this androgen made intracellularly from DHEA in peripheral tissues, the concentrations in 30-35 year-old premenopausal women (n=47) was 0.18 ± 0.07 ng/mL to decrease slightly (22%) to 0.14 ± 0.07 ng/mL in 55-65-year old women (n=377). Serum DHEA, on the other hand, decreased by 56% in the same groups from 4.47 ± 2.19 to 1.95 ± 1.18 ng/mL. Conclusion: It is well known that the availability of DHEA markedly decreases with age while the present data show that its metabolites synthesized intracellularly by the mechanism of intracrinology experience smaller decreases, thus indicating an increasing efficacy with age of the intracrine enzymes which synthesize the intracellular sex steroids from decreasing serum levels of DHEA. In order to minimize the decrease in the intracellular sex steroids which are needed in peripheral tissues, such a mechanism, however, only partially compensates for the large decrease in serum DHEA, especially in the absence of ovarian secretion when the only source of sex steroids in postmenopausal women is DHEA.
The long range objective of our laboratory is to understand the cellular and molecular mechanisms by which signaling pathways and downstream transcription factors coordinate the specification of adrenocortical cells within the adrenal gland. The adrenal cortex is a critical endocrine organ that mediates the mammalian stress response through the robust modulation of steroid output in response to physiologic demand. While it is becoming increasingly clear that paracrine factors are critical to adrenal progenitor cells proliferative maintenance, how endocrine signals are integrated with these inputs to coordinate adrenal steroidogenesis and zonation (differentiation) remains poorly defined. The nuclear receptor Steroidogenic Factor 1 (SF1) is the master regulator of both adrenocortical proliferation and differentiation. In the absence of SF1, the adrenal fails to develop and death results without steroid replacement. The cellular and physiologic context in which SF1 engages in unique transcription programs to mediate these processes is completely unknown. Although previous studies have defined Shh-positive cells embedded in the peripheral cortex as the self-renewing progenitor population that differentiates into functional steroidogenic cells, the paracrine and endocrine mechanisms that regulate this process is undefined. The mechanisms by which these multipotent renewing cells are maintained during adrenal homeostasis will be discussed – specifically how SF1 mediates lineage renewal (self-renewing proliferation) in the progenitor cells, and how paracrine and endocrine signals regulate progenitor cell lineage renewal versus lineage conversions (differentiation) under physiological conditions.
High-throughput detection of adrenocortical carcinoma by liquid chromatography-tandem mass spectrometry – a prospective validation study in 2017 adrenal tumour patients

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Objective: Adrenal masses are discovered incidentally in 3-5% of cross-sectional imaging procedures. A major aim of the diagnostic work-up is the exclusion of adrenocortical carcinoma (ACC), with a reported prevalence of 2-11%. However, current imaging has poor specificity, resulting in frequent unnecessary surgery. A novel diagnostic test, urine steroid metabolomics, combining steroid profiling by gas chromatography-mass spectrometry (GC-MS) and machine learning-based data analysis, has shown promise in improving the specificity of detection of ACC. Methods & Results: Firstly we developed and validated a novel, high-throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for urinary steroid metabolite analysis, cross-validated it to GC-MS and confirmed similar performance in detecting ACC in combination with machine learning using 99 ACA and 45 ACC urines. Next, we undertook a 60-month, prospective, multi-centre validation study involving 14 clinical sites affiliated with the European Network for the Study of Adrenal Tumours (ENSAT). We prospectively recruited 2017 patients with a newly diagnosed adrenal mass (98 (4.9%) ACC) and 24-h urine for high-throughput urine steroid metabolomics. Imaging had a positive likelihood ratio (LR) of 4.80 (95%CI, 4.37-5.26) for detecting ACC, while urine steroid metabolomics provided a LR for a high-risk score of 10.35 (95% CI, 8.72-12.29). A diagnostic strategy consecutively combining tumor diameter, urine steroid metabolomics and follow-up imaging had a post-test ACC probability of 76.4% (95% CI, 67.2-84.1%) for those with a high risk result and 0.3% (0.0-0.6%) for those with a low risk result. This strategy identified 93 of 98 ACCs and only required imaging and urine steroid metabolomics in 488 (24.2%) of 2017 patients. Conclusions: These data indicate superiority of high-throughput urine steroid metabolomics to currently employed routine imaging for the detection of ACC in adrenal incidentaloma.
Androgen Receptor Blockage Induced By DDT Disrupts The Pituitary-gonadal Axis and Results In A Functional Testosterone Deficiency and risk for metabolic disease

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p,p'-DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane] used for indoor residual spraying (IRS) to control malaria vectors, shows estrogenic activity and the persistent metabolite, 1,1-dichloro-2,2-bis(p-chlorophenyl) (DDE), anti-androgenic properties under experimental conditions. No previous study addressed hormonal responses related to living in IRS areas. Circulating isomer and male reproductive hormone concentrations in 535 men (aged 21.8±4 years) living in areas with and without IRS, were determined to explore the association between chronic DDT exposure and male reproductive hormones. Significantly higher t-, f-, bio-T and lower FSH concentrations were observed with both DDT and DDE uptake, while also lower LH occurred with DDT detection only. Men from IRS villages were significantly more likely to have higher t-T, bio-T and E₂ concentrations (OR=2.47 (95% CI:1.19, 3.23); OR 2.48 (95% CI:1.55, 3.97) and OR=2.34 (95% CI:1.33, 4.11)) compared to men from non-IRS villages, after controlling for age, BMI and smoking. Higher circulating T with concomitant conversion to E₂, results in negative feedback to the pituitary, and lower FSH and LH release. These changes may reflect androgen receptor blockage, induced by DDE, rendering these men “androgen-deficient”, thereby impairing spermatogenesis as previously demonstrated. This functional testosterone deficiency may increase risk for the metabolic syndrome, type 2 diabetes as well as coronary vascular disease and warrants studies to determine the long-term health implications of DDT exposed populations.
The glucocorticoid receptor (GR) binds to tens of thousands of genomic binding sites, yet seems to regulate a much smaller number of genes. In a simple scenario, binding of GR would influence the expression of associated genes whereas in the absence of binding, nearby genes would not be affected. In practice however, the link between GR binding and gene regulation is fuzzy, with the majority of binding events apparently not resulting in the regulation of nearby genes. This raises the question: What distinguishes GR binding events that result in the regulation of gene expression from those that do not? We try to answer this question using a variety of approaches, which advanced our understanding in several ways. First, computational approaches indicate that productive GR binding events are more likely when the three-dimensional organization of the genome brings such regions proximal to the promoter of genes. Moreover, we found that promoters of regulated genes share specific sequence features suggesting that only certain promoters are receptive to the regulatory activity of GR-bound regions. Second, using massively parallel reporter assays, we determined the regulatory potential of tens of thousands of GR-bound regions to test if only a subset has an intrinsic regulatory potential. Finally, using genome-editing approaches, we identify single GR-bound recognition sequences that contribute to the GR-dependent regulation of genes. By dissecting an enhancer element with multiple GR recognition sequences and by mutating these, alone or in combination, we uncover that the regulatory potential of the enhancer depends on cooperative interactions between multiple GR binding sites. Together, these studies yield several insights into the molecular mechanisms that discriminate “productive” GR interactions with the genome resulting in gene regulation from “non-productive” binding events.
We and others have recently shown that ERα is expressed in a subset of high grade prostate cancers and is associated with high proliferation rate, poor prognosis and androgen independence. ERα activities are complex: in the cytoplasm ERα can directly stimulate survival signalling at the cell membrane, while in the nucleus ERα activates and represses the transcription of target genes. Here we explore the role of ERα in coordinating transcription and mRNA translation in prostate cancer. Unexpectedly, loss of ERα expression leads to decoupling of transcription and translation events. Namely, mRNAs whose levels are induced by ERα loss exhibit reduced translation efficiency and, vice versa. Such regulation is manifested at the protein level and targets a range of key cellular functions including metabolism and protein synthesis. Our detailed mechanistic assessment revealed that while ERα-regulated microRNA levels contribute to global changes in mRNA levels, translational buffering is explained by changes in ribosome processivity and elongation rate. Thus mRNAs whose levels increase following ERα loss but do not change their association with polysomes, and hence are negatively buffered via translation, use a different set of codons as compared to those mRNAs that are induced and change their association with polysomes. Indeed, ERα modulates the expression of enzymes required for tRNA U34 modifications and their forced expression elevates translational buffering. Overall, we have recently identified a process by which ERα drastically impacts the translation of a subset of mRNAs in cancer cells. We propose that this new regulatory pathway plays a major role in mediating biological effects of ERα in neoplastic tissues. Moreover, our findings have important implications in understanding alterations in gene expression programs following treatment with ERα antagonists.
Androgenic modulation of AR-Vs

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The importance of androgen receptor variants (AR-Vs) is recognized in prostate cancer. Expression of AR-Vs has been proposed as a biomarker for resistance to androgen deprivation therapy for metastatic disease. The aim of the present work, was to investigate the androgenic modulation of AR-Vs expression to provide a better understanding of the molecular regulation of those variants. The C4-2B cell line was chosen as the model for further investigations based on the finding that it expresses detectable levels of AR-Vs. The C4-2B cell line was exposed to low dihydrotestosterone (DHT 10^{-13} M) and high (DHT 10^{-8} M) androgen levels, with or without flutamide. mRNA and protein expression levels were assessed by qPCR and immunohistochemistry, respectively. AR subcellular localization was detected by confocal fluorescence microscopy for AR-FL and AR-V7. To assess the relative cell growth ability of C4-2B in different conditions, sulforhodamine B (SRB) assay was used. In this work, the dynamic changes on AR-FL and AR-Vs were observed under androgen treatment. We demonstrated a negative modulation of AR-FL and AR-Vs by high concentration of androgen (DHT 10^{-8} M). The association of DHT 10^{-8} M with flutamide showed an even greater downregulation effect. Although low levels of androgen (DHT 10^{-13} M) did not change the overall expression of the evaluated receptors, they demonstrated fascinating dynamical changes on cellular localization over time. Treatment with DHT 10^{-13} M induced C4-2B proliferation, which was intensified by the association to flutamide. The results presented here support the assumption that aberrant mRNA processing may underlie prostate proliferation. Our results highlight the contribution of AR-Vs in the dance of androgenic modulation. These findings have implications for a better understanding of the role of AR-Vs in prostate carcinogenesis and, perhaps, indicate new paradigms for treatment of PCa.
Synthetic progestin contraceptives medroxyprogesterone acetate and norethisterone exhibit differential receptor-mediated effects on gene expression and HIV-1 replication

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The synthetic progestin-only injectable contraceptive depo-medroxyprogesterone acetate (DMPA) is widely used in Sub-Saharan Africa, unlike norethisterone enanthate (NET-EN). Epidemiological evidence shows DMPA increases the risk of HIV-1 acquisition by 1.4-fold, while no such association is shown from limited data for NET-EN. Whether MPA and NET have similar effects on HIV-1 acquisition, the biological mechanisms mediating these effects and their dose-response are critical issues for women’s health. MPA is a potent partial to full agonist for the glucocorticoid receptor (GR), unlike NET, while both progestins act as potent progesterone receptor agonists. Cervical tissue, peripheral blood mononuclear cells (PBMCs) and TZM-bl cells were incubated with progestins and infected with HIV-1 infectious molecular clones. HIV-1 replication was determined by p24 assay or Renilla luciferase. Steroid receptor involvement was assessed using the GR antagonist RU486 and siRNA knock down. A combination of qPCR and flow cytometry was used to investigate CCR5 levels and changes in immune cell populations. We show that MPA, unlike NET, significantly increased R5 HIV-1 replication and CCR5 but not CXCR4 levels in cervical explant tissue, PBMCs and TZM-bl cells, at physiologically relevant doses. MPA, unlike NET, also increased activation of T-cells and increased CD4/CD8 ratios in PBMCs. Both increased levels of CCR5 and HIV-1 infection were inhibited by the GR antagonist in all models, as well as by knock-down of the GR in TZM-bl cells. Our results suggest that peak serum levels of DMPA but not NET-EN may increase R5 HIV-1 acquisition and pathogenesis in women at least in part via direct effects on cervical tissue and T-cells via changes in T-cell activity and GR-mediated effects on CCR5 levels. The findings have important implications for choice of progestin for contraception in young women at high risk for HIV-1 infection in Sub-Saharan Africa.
Molecular biochemical characterization of selective glucocorticoid receptor activities of GSK866 analogues with cysteine reactive warheads

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Synthetic glucocorticoids (GC) are the mainstay therapy for treatment of acute and chronic inflammatory disorders. Due to the high adverse effects associated with long-term use, GC pharmacology has focused since the nineties on more selective glucocorticoid ligand binding strategies, classified as selective glucocorticoid receptor agonists (SEGRAs) or selective glucocorticoid receptor modulators (SEGRMs). Following molecular modeling, a novel series of GSK866 SEGRA analogues with covalent warheads was designed and synthesized, analogous to the concept of selective covalent binding kinase inhibitors. Since the off-rate of a covalently binding drug is negligible compared to that of a non-covalent drug, its therapeutic effects can be prolonged and typically, smaller doses of the drug are necessary to reach the same level of therapeutic efficacy, thereby potentially reducing systemic side effects. Different SEGRA GSK866 analogues with cysteine reactive warheads were characterized for GR potency and selectivity in various biochemical and cellular assays. GR- and NFκB-dependent reporter gene studies show favorable anti-inflammatory properes with reduced GR transactivation of two non-steroidal GSK866 analogues UAMC-1217 and UAMC-1218, whereas UAMC-1158 and UAMC-1159 compounds failed to modulate cellular GR activity. These results were further supported by GR immuno-localization and S211 phospho-GR western analysis, illustrating significant GR phosphoactivation and nuclear translocation upon treatment of GSK866, UAMC-1217 or UAMC-1218, but not in case of UAMC-1158 or UAMC-1159. Furthermore, mass spectrometry analysis of tryptic peptides of recombinant GR-LBD bound to UAMC-1217 or UAMC-1218 confirmed covalent cysteine-dependent GR binding. Finally, molecular dynamics simulations, as well as GR-LBD coregulator interaction profiling of the GR-LBD bound to GSK866 or its covalently binding analogues UAMC-1217 or UAMC1218 revealed subtle conformational differences that might underlie their SEGRA properties. Altogether, GSK866 analogues 1217 and 1218 hold promise as a novel class of covalent binding SEGRA ligands for the treatment of inflammatory disorders.
Glucocorticoids (GCs) exert a myriad of metabolic effects mediated predominantly via binding to the glucocorticoid receptor (GR). Sexually dimorphic effects of GC activities provoked our hypothesis that metabolic GC activity is influenced by interaction with sex hormones. Thereto we investigated to what extent metabolic GC signaling is dependent on androgen signaling. Male C57BL/6J mice were exposed to sham-pellets + vehicle treatment (N=6), 20-mg-corticosterone- pellets + vehicle treatment (N=7) or 20-mg-corticosterone-pellets + AR blockage (Enzalutamide, 40 mg/kg/d; N=7) for 2 weeks. Metabolic tissues including gonadal adipose and liver were collected to determine GR-responsive transcript expression via RT-PCR and western blot. Tissue-specific levels of corticosterone and 11-dehydrocorticosterone were determined by LC/MS analysis. Corticosterone induced expression of GR-responsive transcripts Fkbp5, Gilz and Mt2 in adipose and liver (p<0.001). Strikingly, AR blockage diminished corticosterone-induced GR transcriptional activity in both metabolic tissues. LC/MS analysis revealed decreased adipose corticosterone levels upon AR blockage (2.6 vs. 4.3 ng/mg tissue; p<0.01), while enhanced levels of inactive GC metabolite 11-dehydrocorticosterone were found in plasma (3.5 vs. 2.1 ng/ml, p<0.01) and liver (6.0 vs. 3.9 ng/mg tissue, p<0.001). AR blockage reduced expression of 11β-HSD1, converting inactive into active GC, thus explaining the altered proportion corticosterone:11-dehydrocorticosterone. Corticosterone levels correlated with transcriptional output in adipose, i.e. expression of Fkbp5 (R²=0.35) and Mt2 (R²=0.15). In contrast, hepatic corticosterone levels did not correlate with GR transcriptional activity. Our data implies that metabolic GR activity is highly dependent on functional androgen receptor signaling. Diminished GR transcriptional activity in adipose is explained by lowered GC levels, while these do not underlie blunted hepatic GR transcriptional activity. This illustrates that androgen signaling affects GC signaling through different, tissue-specific mechanisms.
Polycystic ovary syndrome (PCOS) affects 10% of all women and is defined by the presence of androgen excess, chronic oligo-anovulation and polycystic appearance of the ovaries. However, PCOS is also a lifelong metabolic disorder, with increased rates of type 2 diabetes, hypertension and cardiovascular disease. Androgen excess is a defining feature of PCOS and generally assessed by measuring serum testosterone (T), which, however, is hampered by very low circulating T in women, challenging even for quantification by mass spectrometry. We have shown that androstenedione (A4), the immediate precursor of T in the classic androgen pathway, is a more sensitive marker of PCOS-related androgen excess and closely correlates with insulin resistance (JCEM 2014;99(3):1027-36). In a human in vivo study, we could show that subcutaneous adipose tissue in women with PCOS drives androgen excess via increased expression and activity of AKR1C3, converting A4 to T, and that this leads to both systemic insulin resistance and lipotoxicity (JCEM 2017;102(9):3327-3339), thereby driving risk factors of type 2 diabetes and fatty liver disease. Insulin-mediated upregulation of AKR1C3 expression completes this vicious circle linking androgen excess and insulin resistance in PCOS. In a further study, we examined the role of the 11-oxygenated androgen pathway, arising from the intra-adrenal conversion of A4 to 11-hydroxyandrostenedione (11OHA4), and ultimately generating the active androgen 11-ketotestosterone (11KT), which binds and activates the androgen receptor with similar potency to T (MCE 2013;377(1-2):135-46). We found in a large cohort of PCOS women that 11-oxygenated androgens comprised the majority of their circulating androgens, pointing to the significance in this pathway in conveying androgen-mediated metabolic risk in PCOS (JCEM 2017;102(3):840-848). We now take this forward by analysing the differential effects of classic and 11-oxygenated androgens on metabolic target tissues and metabolic function in women with PCOS.
Sphingosine-1-phosphate lyase (SGPL1) deficiency is associated with mitochondrial dysfunction

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Background: Loss of function mutations in SGPL1, a key component of sphingolipid metabolism, are associated with accumulation of sphingolipid intermediates giving rise to a multisystemic disease incorporating primary adrenal insufficiency (PAI), progressive renal and neurological disease. Objectives: To investigate impact of SGPL1 deficiency on mitochondrial morphology/function using patient derived dermal fibroblast and SGPL1- knockout HeLa cell lines. Methods: Primary cultures of dermal fibroblasts were established from two patients with SGPL1 deficiency, PAI and renal/neurological compromise (Patient 1 - p.F545del, later onset; Patient 2 - p.S65Rfs*6G, early onset). Mitochondrial architecture was examined by confocal microscopy with volumetric analysis using Z-stack images. Mitochondrial oxidative phosphorylation was measured by Seahorse XFe96 Analyzer in control/patient fibroblasts. RT-qPCR for expression levels of genes regulating mitochondrial fusion and fission (MFN1/2, DRP1).

Results: Total mitochondrial volume in patient fibroblasts and SGPL1-KO-HeLa cell lines vs controls was reduced: (p.F545del; p<0.05; p.S65Rfs*6G; p<0.001), SGPL1-KO-HeLa, p<0.01; n=20. Additionally, the number of fragmented mitochondria was increased in p.S65Rfs*6G vs. control (p<0.0001). The respiratory flux profile of p.F545del fibroblasts was unaltered, however, p.S65Rfs*6G fibroblasts showed a significant reduction in non-mitochondrial respiration, maximal respiration, ATP production and spare respiratory capacity (p<0.05, n=3). Mitochondrial morphology differed; SGPL1-KO-HeLa and p.F545del had hyper-fused mitochondria whereas p.S65Rfs*6G had fragmented mitochondria. MFN1/MFN2 expression were markedly upregulated in SGPL1-KO- and p.F545del fibroblasts (p<0.0001; n=3) while the opposite was seen in p.S65Rfs*6G (p<0.0001). However, DRP1 was uniformly downregulated in SGPL1-KO-HeLa and patient fibroblasts (p<0.0001). Conclusion: Aberrant sphingolipid metabolism leads to disruption of mitochondrial morphology/function. The significantly decreased DRP1 expression suggests an imbalance tilted towards reduced fission. The expression levels of fusion proteins MFN1/2 differed between cell lines; up in SGPL1-KO-HeLa/p.F545del, down in p.S65Rfs*6G fibroblasts. However, in both patient fibroblasts and SGPL1-KO-HeLa cells mitochondrial volume is reduced. Further work is required to characterize mitochondrial effects of SGPL1 deficiency on disease pathogenesis.
20alpha- and 20beta-reduction of glucocorticoids in humans: which role plays CBR1?

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Glucocorticoids play an essential role in human homeostasis by regulating the metabolism, the immune system, and the stress reaction. For inactivation and excretion, both cortisol and cortisone are metabolized to 5alpha-/5beta-dihydro- as well as to 5alpha/5beta-tetrahydro-derivatives. While all these steroids are well known and present in human matrices in high concentrations, the less well described 20alpha- and 20beta-dihydro-derivatives of cortisol and cortisone are detectable only in low, however significant amounts. Up to now, the enzyme(s) responsible for the formation of 20alpha- and 20beta-dihydrocorticosteroids are unknown. One putative candidate enzyme, the human carbonyl reductase 1 (CBR1), recently described to catalyze the conversion of cortisol to 20beta-dihydrocortisol, was closer examined in this respect. After recombinant expression of CBR1 in HEK293 cells, we analyzed the capability of CBR1 to catalyze the conversion of a selected steroid panel with regard to formation of 20beta- and 20alpha-reduced products. Beside cortisol and cortisone we included the substrates progesterone, 11-deoxycorticosterone, corticosterone, 17alpha-hydroxy-progesterone, and 11-deoxycortisol. We separated and analyzed substrates and corresponding C20-reduced products by LC-MS/MS. We observed low, but significant formation of 20beta-dihydro-derivatives of cortisol, cortisone, 11-deoxycorticosterone, and 11-deoxycortisol by CBR1. Interestingly, CBR1 catalyzed the conversion of cortisone more efficiently than the conversion of cortisol, 11-deoxycorticosterone, or 11-deoxycortisol. 20alpha-dihydro-derivatives were not found. Our data indicate that CBR1, formerly mainly known to be involved in exogenous drug metabolism, might be responsible for the generation of 20beta-dihydrocortisone in humans. Whether CBR1 is the only source for 20beta-dihydrocortisone formation in humans and which enzyme(s) produce(s) 20alpha-dihydrocortisone, remains elusive.
Intracrine activation of 11-oxygenated androgens by AKR1C3 is increased by HSD11B1 inhibition

Recent studies have shown that 11-ketotestosterone (11KT) is the most abundant circulating androgen in women. While the enzymes involved in its biosynthesis are known, the major sites of production remain unclear. The androgen activating enzyme aldo-ketoreductase 1C3 (AKR1C3) is highly expressed in adipose tissue and catalyses the conversion of 11-ketoandrostenedione (11KA4) to 11KT significantly more efficiently than that of androstenedione (A4) to testosterone (T), thus suggesting a central role of adipose tissue in 11KT activation. However, 11ß-hydroxysteroid dehydrogenase type 1 (HSD11B1) is also highly expressed in adipose tissue, catalysing the conversion of 11KA4 back to 11β-hydroxyandrostenedione (11OHA4), therefore potentially limiting 11KT production by AKR1C3. We therefore set out to determine adipose-specific 11KT production and to determine if pharmacological inhibition of HSD11B1 results in a shift towards 11-oxygenated androgen activation. Using primary human female subcutaneous and omental adipose tissue, we found that adipose tissue preferentially activated 11KT in agreement with the in vitro activity of AKR1C3. Ex vivo HSD11B1 inhibition resulted in impaired reduction of 11KA4 to 11OHA4 and enhanced conversion of 11KA4 towards the potent androgen 11KT, while having no discernible effect on conversion of A4 to T. Serum and urinary steroid profiling in 31 women (age 31.2±6.9, BMI 39.2±12.6) treated for 12-weeks with a HSD11B1 inhibitor demonstrated significant inhibition of HSD11B1 activity with reduced systemic glucocorticoid activation. Concomitantly, 11-oxygenated androgen metabolism was altered: serum 11OHA4 and 11KA4 were significantly increased as a result of increased adrenal ACTH stimulation, while the ratio of urinary 11-oxygenated androgen metabolites shifted significantly towards the active keto-derivatives. We conclude that adipose tissue AKR1C3 makes a significant contribution to the peripheral activation of 11-oxygenated androgens, an effect that is enhanced by HSD11B1 inhibition. The metabolic consequences of increased 11-oxygenated androgens should therefore be taken into account when considering this treatment to reduce local cortisol exposure.
The metabolism of adrenal C11-oxy C21 steroids to C11-oxy androgens in the backdoor pathway

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Context: 21-hydroxylase deficiency (21OHD), polycystic ovarian syndrome (PCOS) and prostate cancer (PCa) are characterized by elevated levels of adrenal androgens and C11-oxy steroids including 11β-hydroxyandrostenedione (11OHA4), 21-deoxycortisol (21dF) and low levels of 11β-hydroxyprogesterone (11OHP4). These steroids are substrates for 11βHSD2 yielding C11-keto derivaves. 11OHA4 acts as a precursor to more potent androgens such as 11-ketotestosterone and 11-keto-dihydrotestosterone (11KDHT). Elevated C11-oxy C19 and C21 steroid levels have been idenfied in serum profiles of 21OHD, PCOS and PCa patients including 11OHP4, 11-ketoprogesterone (11KP4), progesterone, 17-hydroxyprogesterone and 21dF. The latter two are metabolized in the backdoor pathway leading to DHT and 11KDHT production. Objectives: To investigate the biosynthesis of 11OHP4 and the metabolism of C11-oxy C21 steroids by enzymes of the backdoor pathway and their contribution to androgen levels. Methods: Enzymatic assays investigating the biosynthesis and conversion of C11-oxy C21 steroids were done using transfected HEK-293 cells expressing the relevant enzymes. The metabolism of C11-oxy steroids was assayed in human carcinoma cells. Steroids were analyzed using Q-TOF MS and/or UPC2-MS/MS. RESULTS: The 11β-hydroxylaon of progesterone (1 µM) by CYP11B1 and CYP11B2 yielded 0.6 and 0.5 µM 11OHP4 which was metabolized by 11βHSD2 to 11KP4. 11OHP4 and 11KP4 are rapidly reduced by SRD5A and subsequently AKR1C2. These 3α,5α reduced metabolites are excellent substrates for both 17α-hydroxylase and 17,20-lyase activity of CYP17A1, suggesting minimal repositioning of the C11-oxy substrate in the active pocket compared to the natural substrates. Metabolism of C11-oxy C21 steroids in human cell models showed evidence for the biosynthesis of C11-oxy C21 steroids and C11-oxy androgens. Conclusions: This study provides evidence for the in vitro metabolism of C11-oxy C21 steroids to C11-oxy androgens by steroidogenic enzymes. These findings highlight the necessity to include these steroids in future steroid profile studies associated with adrenal-linked hyperandrogenic endocrine diseases and their role in hormone/receptor-driven diseases.
Adrenal aging, gene mutations and primary aldosteronism

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Primary aldosteronism (PA) represents the most common cause of secondary hypertension, but little is known regarding its adrenal cellular origins. Recently, small foci of cells that express the enzyme aldosterone synthase (CYP11B2) were described and called aldosterone-producing cell clusters (APCCs). APCCs were found in normal adrenals and adrenals with adjacent aldosterone-producing adenoma (APA), suggesting that APCCs express CYP11B2 in a renin-independent manner. Broad use of next generation sequencing for APA DNA demonstrated the presence of mutations in genes encoding ion channels/pumps that alter intracellular calcium homeostasis and cause renin-independent aldosterone production through increased CYP11B2 expression. We have studied APCCs in normal and pathologic adrenals with the hypothesis that these CYP11B2-expressing cells have aldosterone-stimulating somatic gene mutations. APCCs have been studied in over 200 adrenals from autopsy and renal donor patients, as well as in adrenals from patients with bilateral hyperaldosteronism (BHA). APCCs have been studied with regard to gene expression profiles, somatic mutation status and their changes with aging. The APCC transcriptome was most similar to zona glomerulosa (ZG) but with an enhanced capacity to produce aldosterone. APCCs harbored APA-related mutations as determined by targeted next generation sequencing. These mutations were not present in DNA from adjacent normal adrenal tissue. Aldosterone driver mutations were identified in the voltage-dependent calcium channel, L-type, α1D-subunit (CACNA1D) and ATPase, Na+/K+ transporting, α1-polypeptide (ATP1A1), which were similar to those seen in APA. With age, the APCC area and number of APCC with mutations increases. Finally, APCC increased in adrenals from patients with BHA, the most common cause of primary aldosteronism. Overall, we have found several significant findings: (i) APCCs and somatic aldosterone-driver mutations are often seen in normal adrenals, (ii) adrenal APCCs/somatic mutations increase with aging, and (iii) Aldosterone-driver mutations and APCC are much higher in BHA adrenals. These results provide molecular support that somatic genes mutations are the primary contributor to adrenal dysregulated aldosterone production.
Are adrenal C11-oxy C19 and C11-oxy C21 steroids relevant in benign prostatic hyperplasia and prostate cancer?

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This study investigated C11-oxy C19 and C11-oxy C21 steroid levels in benign prostatic hyperplasia (BPH) and prostate cancer (PCa) patients. The contribution of testosterone to androgen levels in normal and cancerous prostatic tissue is well documented. However, the contribution of adrenal hormones to the development of PCa has also been highlighted as C11-oxy C19 steroids contribute potent androgens to the PCa microenvironment through the 11β-hydroxyandrostenedione (11OHA4) pathway. Adrenal C11-oxy C21 steroids are also role-players as they provide androgens to the prostate through the backdoor pathway. These adrenal C11-oxy steroids warrant further investigation in the context of BPH and PCa. Here, we report on the metabolism of C11-oxy C19 and C11-oxy C21 steroids in BPH and PCa and quantify circulating C19 and C21 steroids in patients utilizing ultra-performance convergence chromatography tandem mass spectrometry. C11-oxy C19 steroids were efficiently metabolized in C4-2B and BPH-1 cells, producing 11-ketotestosterone (11KT), 11-ketodihydrotestosterone (11KDHT) and 11β-hydroxyandrostosterone (11OAST). In BPH tissue, C11-oxy C19 steroids were present at significantly higher levels compared to C19 steroids, while 11-hydroxyprogesterone (~27 ng/g), 11-ketoprogesterone (11KPROG; ~130 ng/g) and 11-ketodihydroprogesterone (~282 ng/g) were the predominant C11-oxy C21 steroids present. In circulation, 11OHA4 (~19 nmol/L) and 11KDHT (~12 nmol/L) were the dominant C11-oxy C19 steroids, while 11KPROG (~74 nmol/L) and dihydroprogesterone (~23 nmol/L) were also present. In PCa tissue, 11KPROG (~371.6 ng/g) and 11OHA4 (~23.1 ng/g) were predominant, together with 11KT (~12.7 ng/g) and 11KDHT (~11.3 ng/g). High circulatory levels of 11KPROG (~1.7 µmol/L) were quantified in PCa patients, together with: 11OHA4 (~32.8 nmol/L) > 11OAST (~27.6 nmol/L) > 11KT (~11.8 nmol/L) > 11KDHT (~10.8 nmol/L). This investigation highlights the contribution of adrenal steroids to the BPH and PCa microenvironment providing potential insight into disease progression and identifies novel C11-oxy C21 steroids and the capacity of prostate cells to produce potent androgens.
Obesity is a known risk factor for both pre- and postmenopausal breast cancer, however, the risk is greater in postmenopausal women. This may be because menopausal transition is also associated with weight gain and central adiposity, as well as elevated levels of adipokines. Although the detailed mechanisms through which adiposity promotes breast cancer development is still largely unknown, numerous studies have suggested that adipokines are key metabolic links between obesity and breast cancer. Hormone therapy (HT), used by many postmenopausal women to alleviate the symptoms of menopause, have been linked to increased breast cancer risk. Importantly, studies investigating whether there is any direct association between obesity, HT and breast cancer are lacking. The primary objective of this study was therefore to compare the activities of selected hormones used in HT on steroid biosynthesis and adipokine production in adipose tissue, and how these activities influence breast cancer biology. Treatment of both T47D and MDA-MB-231 human breast cancer cells with differentiated 3T3-L1 murine adipocyte conditioned media resulted in a significant increase in invasion of these breast cancer cell lines. Investigating the effects of specific adipokines showed that leptin increased proliferation of the estrogen receptor positive (ER+) T47D cell line, whereas proliferation was decreased in the ER negative (ER-) MDA-MB-231 cell line. We also found that treatment with the adipokine plasminogen activator inhibitor 1 (PAI-1) significantly increased proliferation of both ER+ and ER- breast cancer cell lines, which was enhanced in the presence of estrogen. Interestingly, co-treatment with the 4th generation progestin drospirenone and PAI-1 significantly increased cell growth compared to drospirenone alone. These results suggest that adipokines associated with obesity, together with hormones used in HT, directly influence breast cancer cell proliferation and invasion.
Comparison of the antioxidant activity of genistein and quercetin on ROS generation in different cell lines

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Isoflavonoids and flavonoids are widely known as antioxidants. However, at some concentrations they can exert an opposite (prooxidation) activity. Aim of this study was to compare the effects of non-toxic concentrations of isoflavone and phytoestrogen genistein and flavonoid quercetin on the generation of ROS induced by hydrogen peroxide in NIH/3T3 and MCF-7 cell lines. Non-toxic concentrations of genistein and quercetin were established by MTT assay on mouse embryonic fibroblasts NIH/3T3 and human breast cancer cells MCF-7. Subsequently both cell lines were incubated with eight different concentrations of genistein and quercetin (0.01, 0.1, 1, 1.25, 2.5, 5, 7.5, and 10 µM) for 1 h. The ROS generation was measured using the DCFH-DA method with H₂O₂ as a prooxidant agent. The DCF fluorescence was measured at 480/530 nm. All tested concentrations of quercetin induced significant antioxidant activity in MCF-7 cell line. However, only concentrations between 2.5 µM and 10 µM displayed significant antioxidant activity in NIH/3T3 cell line. The lower tested concentrations of quercetin had prooxidant effect. All tested concentrations of genistein exhibited weak antioxidant activity in both cell lines. Our results indicate that antioxidant activity of quercetin is concentration and cell-dependent while genistein appears to be only a weak antioxidant. This study was supported by Slovak Research and Development Agency (grant no. APVV-16-0207) and Scientific Grant Agency VEGA (grants No. 1/0561/18 and 1/0359/18).

Keywords: isoflavonoid; flavonoid; antioxidant activity; reactive oxygen species.
Mortality from cancer is predominantly due to systemic dissemination of tumour cells. Numerous studies have addressed the question of how tumours metastasise but few have asked why do they metastasise? What makes cells detach adhesions from their neighbours, force their way through a complex meshwork of extracellular structure to enter a vascular system of hostile antibodies and immune killer cells? It is a widely held view that hypoxic conditions facilitate metastasis, through glycolytic activation and extracellular acidification, yet these cells are buried deep within the tumour far from the vascular network. We have proposed a model, based on in vitro observations, that suggests tumour metastasis as an active escape from a hostile environment. To study the mechanisms of therapeutic resistance to endocrine agents, we have established breast cancer cell lines that reflect precisely this behaviour and display the means to effect an escape from an imposed alkaline environment that will otherwise prove fatal to their survival. We observe a protective reaction to increased external pH that minimises extracellular contact by cellular contraction and rounding (resembling a pre-apoptotic response), with development of extensive locomotive membranous blebs for cellular migration. Restoration of pH 7.4 reverses this transformation with re-arrangement of cortical actin and a flow of associated proteins including integrin α2, FAK and JAM-1. Pre-treatment with cytochalasin-D or inhibitors of Rho or MLCK prevents both contractolation and bleb formation at high pH. Suppression of bleb formation can also be achieved with Na+/K+ flux inhibitors most likely through their associated intracellular signalling. Gain of endocrine independence and transition to a mesenchymal form facilitates this behaviour, the further study of which will provide indications of how such cancer cells escape into the circulation. Inhibition of blebbling, which is specific to these cells, could be an effective means of retarding metastasis, and therefore cancer mortality.
Chemopreventative effect of SM6Met-a phytoestrogenic compound from honeybush, on LA7-induced mammary tumor in rats

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Background: Breast cancer is the leading cause of cancer-related deaths in women. Chemoprevention of breast cancer with plant extracts might be a less toxic approach in halting cancer progression. SM6Met is a well-characterized phytoestrogenic extract of honeybush (Cyclopia subternata) with reported selective estrogen receptor subtype activity. There is no information on the estrogen sensitivity of the relatively new orthotopic model of LA7 cell-induced mammary tumors or the chemopreventive potential of SM6Met on LA7-induced tumor growth.

Aim: The potential chemopreventative and side-effect profile of SM6Met on LA7 cell-induced tumor growth was evaluated, as was the effects of estrogen (17β-estradiol) and standard-of-care (SOC) endocrine therapies, such as tamoxifen, letrozole and fulvestrant. Results: Progressive tumor growth induced by LA7 cells was observed in control group until day 10 post tumor induction. SM6Met suppressed tumor growth to the same extent as tamoxifen, while letrozole, but not fulvestrant, also showed substantial anti-tumor effects. Short-term 17β-estradiol treatment reduced tumor growth, whereas tumor promoting effects were observed during long-term treatment. Tumor induction caused a marked elevation in serum markers of liver injury as observed in rats treated with tamoxifen, letrozole and long-term 17β-estradiol. However, SM6Met treatment did not increase the levels of liver injury biomarkers. Conclusion: The mammary tumor suppressing effect of SM6Met is comparable to the effect of tamoxifen, without eliciting the negative side-effects of tamoxifen on the liver. Furthermore, the responsiveness of LA7-induced tumors to estrogen and SOC endocrine therapies suggest a potential use of this model in evaluating therapies for hormone responsive breast cancer.
Deregulation of mouse and human 11beta-hydroxysteroid dehydrogenase type 1 and type 2 in colon cancer

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Introduction & Aim: Cortisol and corticosterone are the principal endogenous glucocorticoids (GCs), which are released by adrenal glands. However, there is increasing evidence that several peripheral tissues can modulate the local GCs concentration by extra-adrenal synthesis of glucocorticoids and/or by local regeneration of biologically inactive glucocorticoids cortisone and 11-dehydrocorticosterone to cortisol and corticosterone. The interconversion between cortisol/corticosterone and inactive cortisone/11-dehydrocorticosterone is catalyzed by 11beta-hydroxysteroid dehydrogenase type 1 (11HSD1) and type 2 (11HSD2). 11HSD1 predominantly reduces cortisone/11-dehydrocorticosterone to cortisol/corticosterone, whereas 11HSD2 is an oxidase, which inactivates cortisol/corticosterone to cortisone/11-dehydrocorticosterone. Despite the abundance of both isoforms of 11HSD in colon, little is known about the importance of 11HSD1 and 11HSD2 during tumorigenesis.

Materials and methods: The neoplastic and non-neoplastic tissue samples were obtained from (1) murine healthy colon and colorectal tumors of mice treated with azoxymethane and dextran sodium sulfate and (2) patients with colorectal adenocarcinomas (CRC) or patients who underwent polypectomy. The expression of 11HSD1 and 11HSD2 was measured by real-time PCR, Western blotting and immunohistochemistry.

Results: In mouse tumor, the expression of 11HSD1 mRNA and protein was upregulated, whereas 11HSD2 downregulated and these changes were accompanied by changes in the expression of C/EBP but not RelA and Egr-1 transcription factors. Similar to murine tumors, 11HSD1 mRNA was increased and 11HSD2 mRNA decreased in human CRC and this decrease was observed already in the earlier stages of neoplastic transformation such as adenomas with low and high grade dysplasia; but the levels of 11HSD1 mRNA were not changed.

Conclusions: The results demonstrate that 11HSD1 and 11HSD2 are dysregulated during the development of colorectal polyps and their transformation into CRC, which might have a functional significance. The study was supported by Czech Science Foundation.
From apple leaves to steroid dehydrogenase inhibitors as potential anticancer lead structures – a virtual screening success story

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Objectives: Malus sp. (apple) is amongst the widest cultivated fruit crops worldwide. As a side product of apple farming, a lot of biomass in terms of leaves, peel, bark and roots is generated every year. This material is particularly rich in secondary plant metabolites, which may constitute valuable compounds for medicinal use. The aim of this study was to find molecular targets for the most abundant chemical class of apple leave constituents, the dihydrochalcones. For efficient biological activity identification, we combined virtual target fishing with in vitro screening methods. Methods: A combined in silico approach was employed for biological activity prediction. We used parallel pharmacophoric profiling along with publicly available tools such as 2D/3D similarity search and molecular docking. Targets were ranked according to consensus predictions. Top ranking targets were tested by in vitro inhibition assays. Results: The virtually predicted targets were mostly involved in steroid hormone signaling (estrogen receptors) or metabolism (hydroxysteroid dehydrogenases (HSDs) as well as aromatase). Biological testing partly confirmed these calculations: while 11β-HSDs were not affected, 17β-HSD 3, 4 and 5 inhibition was confirmed for phloretin and several of its analogues. While type 3 and 5 inhibition may be beneficial for the treatment of steroid related diseases, e.g., castration-resistant prostate carcinoma or endometriosis, type 4 inhibition may lead to adverse effects due to impaired lipid degradation. The selectivity profile of the active compounds must therefore be improved. Conclusion: Using a combined in silico – in vitro workflow, we identified dihydrochalcones as novel starting points for anticancer drug development. Chemical optimization studies and biological testing will be conducted in the future to further customize the compounds activities and selectivities.
Circulating steroid levels in prostate cancer patients on hormonal therapy: how low can you go?

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Objective: Steroid hormones are the principal target of prostate cancer (PC) treatment by reducing circulating androgen levels or inhibiting local androgen action. Yet, the full scope of steroidogenic changes these drugs impose on the circulating steroid metabolome of PC patients have not been completely mapped. As large plasma collections of PC patients were collected in CellSave tubes, we explored whether these samples are suitable for steroid metabolome measurements.

Method: Blood was collected in BD SSTII Advance tubes (serum), BD vacutainer Barricor and CellSave Preservative Tubes from 10 healthy volunteers and 10 castration-resistant PC (CRPC) patients on different hormonal treatments. The levels of 20 steroid hormones of the canonical and alternative androgen, mineralocorticoid and glucocorticoid synthesis pathways were measured by liquid chromatography-tandem mass spectrometry.

Results: For most steroid hormones limits of detection were 25-300 pM, but these were higher for DHEA and DHT (0.8 nM). In the control subjects, no significant differences were detected between samples collected in serum or Barricor. Lower values were observed in CellSave compared to serum tubes for testosterone (-8.8%), cortisol (-12.8%) and cortisone (-9.75%). Correlation between the measurements was high (R² ≥ 0.92). In CRPC patients, testosterone values ranged from 130-280 pM for androgen-deprived (ADT) patients, 150–820 pM in patients on ADT+antiandrogens (ADT+AA) and 35–76 pM in abiraterone and/or prednisone (ADT+AP/P) treated patients. For androstenedione values were 0.37–2.55 nM in ADT/ADT+AA-treated patients and <0.1nM for ADT+AP/P-treated patients. Cortisol levels were much lower in ADT+AP/P patients (0.87–13.3 nM) than in other patients (217–517 nM) and 11-deoxycortisol and cortisone were similarly reduced.

Conclusion: In this pilot study using different matrices it was possible to quantify most circulating steroids in CRPC patients on first- and second-line hormonal therapy. After further optimization for some steroids, these levels could be explored as biomarkers for efficacy of and resistance to hormonal treatment.
Tumor microenvironment and its pharmacological modulation in estrogen positive breast cancer model parallels to skin wound healing

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Objective: Breast cancer is the second most frequently diagnosed cancer and the leading cause of death among woman in developed countries. Currently, it is suggested that the inhibition of biological pathways that are associated with the tumor microenvironment may be critical to the treatment of several cancers. In present study, we define key signaling molecules involved in the formation of the tumor microenvironment. Additional attention will also be given to show whether targeted modulation of these regulators promote wound healing.

Methods: Whole-genome transcriptome profiling was performed in cancer associated fibroblasts isolated from different tumors (including breast cancer) and clinical samples of breast cancer. Furthermore, the effect of cancer associated fibroblasts on the differentiation status of epithelial cells was also realized using in vitro techniques.

Results: Whole-genome transcriptome profiling of in vitro experiments and clinical samples revealed that for example interleukins (IL)-6, IL-8, chemokine CXCL-1, galectin-1, vascular endothelial growth factor, and selected proteins of the extracellular matrix (e.g. fibronectin) do have similar regulation during wound healing and tumor growth. Furthermore, these molecules also significantly modulate the differentiation status of epithelial cells in vitro and thus are potent modulators of wound healing.

Conclusion: Previously published and here obtained data demonstrate remarkable similarities between the tumor and wound microenvironments. In particular, components of ECM, growth factors, cytokines/chemokines, and galectins are potent modulators of cancer growth and spreading. Therefore, specific manipulations of cancer stroma can have important therapeutic consequences. Moreover, better understanding of cancer cell-stroma interaction can help to improve wound repair. The present study was supported in part by the Agency for Science and Research (under the contract Nos. APVV-16-0446, APVV-16-0207 and APVV-14-0731).
Biological and clinical implications of aromatase inhibitors for neoadjuvant therapy in postmenopausal women with estrogen receptor positive breast cancer

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Objective: Androst-5-ene-3β, 17β-diol (ADIOL) possesses estrogenic properties. Here we report the efficacy of aromatase inhibitors (AIs) and the influence of letrozole on breast cancer tissue levels and plasma levels of estrone (E₁), estradiol (E₂), estrone sulfate (E₃S), ADIOL, and androstenedione (AONE) in postmenopausal women undergoing neoadjuvant therapy for estrogen receptor positive breast cancer. Methods: Thirty-eight postmenopausal patients with stage I (n=3), IIA (n=16), IIB (n=6), IIIA (n=2), IIIB (n=10) and IIIC (n=1) breast cancers were treated with anastrozole, letrozole or exemestane as neoadjuvant treatment. Plasma and breast cancer tissue samples were obtained from 16 patients before and after 4 months of neoadjuvant therapy with letrozole. Results: One of 38 patients had a complete response, 17 had a partial response and 20 had stable disease. All treatments were well tolerated. Suppression of Ki67 after treatment was significantly greater in responding tumors than in non-responding tumors (P=0.009). The mean breast cancer tissue levels of E₁, E₂, E₃S, ADIOL, and AONE at baseline were 470.1, 328.8, 1134.3, and 4816.2 fmol/g tissue, respectively. After neoadjuvant therapy, the levels of E₁, E₂, E₃S, ADIOL, and AONE were 31.7, 10.2, 4.5, 1509.1, and 4888.1 fmol/g tissue, respectively. The mean plasma levels of E₁, E₂, E₃S, ADIOL, and AONE at baseline were 392.5, 24.2, 1843.4, 944.0, and 2131.4 pmol/l, respectively. After therapy, the levels of E₁, E₂, E₃S, ADIOL, and AONE were 1.4, 2.1, 11.5, 1000.1, and 2680.5 pmol/l, respectively. Letrozole significantly suppressed estrogens. Although plasma and tissue ADIOL levels were high after treatment, there was no progress disease in the 16 cases. Conclusions: Neoadjuvant AIs therapy provided satisfactory efficacy and safety profiles. The activity of AIs was correlated with significantly reduced estrogens levels, but not with ADIOL levels.
Polypharmacological sensitisation of heterogenous multidrug resistant cancers by the steroidal withanolide Withaferin A

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Withaferin A (WA) isolated from Withania somnifera (Indian ginseng or Ashwagandha in Ayurvedic medicine) has recently become an attractive steroidal phytochemical under investigation in various preclinical studies for cancer treatment. Pharmacological levels of WA trigger clinically relevant anticancer effects specific to triple negative breast cancer cells, glucocorticoid therapy resistant multiple myeloma and therapy resistant neuroblastoma. Genomewide DNA methylation, gene expression and pathway enrichment analysis revealed epigenetic suppression of multiple cancer hallmarks associated with cell cycle regulation, cell death, cancer cell metabolism, cell motility and metastasis. By means of peptide array based tyrosine phosphopeptide fingerprinting and kinome activity profiling, we found that WA inhibits various redox sensitive tyrosine kinases which are hyperactivated in hormone therapy resistant cancer cells, including TEC, BTK and HCK kinases. Furthermore quantitative chemoproteomics approaches revealed that the superior cancer therapy efficacy by WA is a consequence of simultaneous targeting of various cellular stress response pathways like proteasome degradation, lipid peroxidation, autophagy and unfolded protein stress responses which altogether exceed the cellular redox homeostasis capacity and promote noncanonical ferroptosis. Nano-targeting of WA further allows systemic application and suppressed tumor growth due to an enhanced accumulation at the tumor site. Collectively, our data propose a novel therapeutic strategy to efficiently kill heterogenous multidrug resistant cancer cells by a natural plant derived steroid WA. Further reading: i) Oncotarget. 2017 Jun 20;8(25):40434-40453. ii) J Proteomics. 2018 May 15;179:17-29. doi: 10.1016/j.jprot.2018.02.013. iii) J Clin Invest. 2018 Jun 25. pii: 99032. doi: 10.1172/JCI99032.w.
Androgen receptor (AR) as AR variants (AR-Vs) play an important role in the molecular changes involved in the pathophysiology of benign prostatic hyperplasia (BPH) and hormone-naïve PCa. The objective of this work was to identify the presence of AR isoforms in benign tissue and primary PCa, and to evaluate the possible association with tumor aggressiveness and biochemical recurrence in primary PCa. The mRNA levels of full length AR (AR-FL) and AR-Vs (AR-V1, AR-V4 and AR-V7) were measured using RT-qPCR. All investigated mRNA targets were expressed in both BPH and PCa. AR-FL mRNA levels were similar in both groups. AR-V4 mRNA expression showed higher levels in BPH, and AR-V1 and AR-V7 mRNA expression were higher in PCa. The ratio of AR-V1/AR-FL was associated with a higher risk of biochemical recurrence. These results support the assumption that these constitutively active isoforms of AR are involved in the pathophysiology of primary PCa and BPH. The identification and functional characterization of differentially expressed molecules between normal and tumoral tissues are fundamental steps to achieving a better understanding not only the carcinogenic process and the development of new anti-tumoral strategies, but also a better understanding of the processes that govern proliferation in benign tissues such as BPH.
Hormone therapy (HT), which includes administration of either estrogen alone, or estrogen combined with a progestin, is used by millions of women to combat the array of symptoms associated with menopausal transition. However, clinical trials have suggested that progestins, used in HT to prevent estrogen-induced endometrial hyperplasia, are associated with an increased risk of developing invasive breast cancer. Considering that all progestins available for clinical use have not been investigated, it may be that some progestins do not increase breast cancer risk. Progestins, all differentiated by structure, were designed to act via the progesterone receptor (PR) and elicit similar responses to that of natural progesterone. Numerous studies have also implicated the PR in breast cancer development, yet seldom distinguish between the two functionally distinct isoforms, PR-A and PR-B. This is important as PR-A is overexpressed, relative to PR-B, in breast cancer. Thus, this study directly compared the activity of a number of different progestins via the individual PR isoforms. Specifically, we investigated effects of progestins on the regulation of genes involved in breast cancer development and progression as well as hallmarks of cancer, via the individual PR isoforms. Moreover, we investigated whether the overexpression of PR-A relative to PR-B influenced the above-mentioned responses. The results showed that effects of the progestins on selected genes involved in breast cancer biology, on cell proliferation, apoptosis, migration, invasion, adhesion and anchorage-independent growth, were dependent on the specific progestin and PR isoform. Overexpression of PR-A relative to PR-B, in the presence of most progestins, inhibited the above-mentioned physiological processes involved in breast cancer development and progression, suggesting that enhanced PR-A expression may be a positive prognostic marker for breast cancer. The findings of this study also emphasise the importance of investigating effects of the individual progestins, but also effects via the individual PR isoforms.
The regulation of the progesterone receptor isoforms by progestins and the glucocorticoid receptor

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Progestins were designed to mimic the action of the endogenous hormone, progesterone (P4) via the progesterone receptor (PR), which has two isoforms, PR-A and PR-B. Progestins are administered as hormonal contraceptives and hormone replacement therapy (HRT) treatments. However, currently their transcriptional actions via PR-A and -B have not been well-characterised. It is established that steroid receptors (SRs) interact and influence each other's transcriptional response. Importantly, the modulation by the ubiquitous SR, the glucocorticoid receptor (GR), of PR transcriptional activity has not been investigated even though these receptors are present in one cell simultaneously. The first objective was to determine the biocharacter, potency (EC50) and efficacy of progestins via the PR isoforms, by promoter-reporter assays. The second objective was to investigate PR transcriptional activity in the presence and absence of the GR. This was carried out by siRNA-treatment specific for the GR followed by promoter-reporter assays or quantitative real-time PCR. The experiments were performed in PR-A or -B stably-expressing breast cancer cell lines or U2OS cells transiently transfected with PR, while all these cells express endogenous GR. Seven widely-used progestins were full agonists for transactivation via both PR isoforms. All progestins were more potent via PR-A than PR-B, but significantly more efficacious via PR-B. After GR-specific siRNA-treatment, promoter-reporter assays showed that the GR modulated PR-B activity. The most significant change was seen with efficacy where ligands specific for the PR were more efficacious when the GR was absent. These data relate to current therapeutics designed to target only one SR such as contraceptives designed to only target the PR. These data show that progestins will be significantly less efficacious in cells with higher GR levels and suggest that modulation of relative GR and PR levels in vivo may have implications for efficacy of contraceptives and HRT in a target cell-specific manner.
Differential gene expression and metabolism of synthetic progestins via the glucocorticoid receptor ex vivo and in vivo

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Progestins are the main component of hormonal contraceptives which are widely used in Sub-Saharan Africa. Progestins are synthetic analogues of progesterone (P4), which were designed to act only via the progesterone receptor (PR). However, some progestins exhibit off-target binding with other steroid receptors such as the glucocorticoid receptor (GR). The metabolism of contraceptive progestins Medroxyprogesterone acetate (MPA), Etonogestrel (ETG), Levonogestrel (LNG) and Nestorone (NES) was investigated in various biological systems to identify a potential correlation between metabolism, off-target binding and transcriptional activity via the GR. Potencies and efficacies for gene expression were determined on endogenous GR-responsive genes in a primary model and cell line. Progestin metabolism was quantified in a primary model and cell line utilizing UHPSFC-MS/MS. All progestins but NET induced GILZ expression in COS-1, with only DEX, ETG, and LNG being significantly metabolized. Differential gene expression was observed with DEX, MPA and NES in PBMC’s with only DEX and P4 being significantly metabolised. DEX, MPA and NES exhibited the same metabolism and transcriptional activity across the two model systems. The transcriptional activity of ETG, LNG and P4 via the GR, as well as their metabolism, seems to be cell type, gene, and ligand specific. The parallel comparison of currently available progestins indicates that there is no correlation between metabolism, off-target binding and transcriptional activity via the GR.
Nuclear Receptor Signalling

Poster 16

Semi-synthetic sapogenin exerts neuroprotective effects by skewing the brain ischemia reperfusion transcriptome towards inflammatory resolution

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Stroke represents one of the first causes of mortality and morbidity worldwide. We evaluated the therapeutic potential of a novel semi-synthetic spirosteroid sapogenin derivative "S15" in a transient middle cerebral artery occlusion (tMCAO) focal ischemia model in rat. S15-treated rats had significantly reduced infarct volumes and improved neurological functions at 24h post-reperfusion, compared with ischemia. Corresponding gene expression changes in brain were characterized by mRNA sequencing and qPCR approaches. Next, we applied geneset, pathway and transcription factor motif enrichment analysis to identify relevant signaling networks responsible for neuronal damage upon ischemia-reperfusion or neuroprotection upon pretreatment with S15. As expected, ischemia-reperfusion brain damage strongly modulates transcriptional programs associated with immune responses, increased differentiation of immune cells as well as reduced (cat)ion transport and synaptic activity. Interestingly, S15-dependent neuroprotection regulates inflammation-associated genes involved in phagosome specific resolution of tissue damage, chemotaxis and anti-inflammatory alternative activation of microglia. Altogether our transcriptome wide RNA sequencing and integrated pathway analysis provides new clues in the neuroprotective properties of a novel spirosteroid S15 or neuronal damage in rat brains subjected to ischemia, which opens new perspectives for successful treatment of stroke.
The accurate identification and quantification of steroid panels is a vital aspect of clinical steroid research and diagnostics. Urine and serum steroid panels are currently routinely analysed using gas chromatography-mass spectrometry (GC-MS) and ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS), respectively. While, both GC-MS and UHPLC MS/MS have their specific advantages, neither are without limitation. The development of ultra-high performance supercritical fluid chromatography tandem mass spectrometry (UHPSFC-MS/MS) instruments potentially overcome these limitations, while at the same time combining the strengths of both GC-MS and UHPLC-MS/MS. We therefore present methods for the separation of both serum and urinary steroid analytes by UHPSFC-MS/MS and compare these methods to routine UHPLC-MS/MS and GC-MS methods, respectively. These panels include the urine and serum metabolites of the recently discovered 11-oxygenated adrenal androgen metabolites that have not yet been included in routine steroid analyses but have been identified as major contributors in androgen dependent diseases. We show that the UHPSFC-MS/MS workflow requires less sample preparation than GC-MS while offering high throughput runs comparable to UHPLC-MS/MS. This, together with increased sensitivity and resolution despite lower injection volumes make this a valuable tool for the analyses of samples in the clinical setting. Taken together, our results highlight the potential of UHPSFC-MS/MS for routine analyses in both the research and clinical laboratory.
A whole new world for 11β-hydroxysteroid dehydrogenase: the metabolism of adrenal C11-oxy C19 and C11-oxy C21 steroids

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11β-hydroxysteroid dehydrogenases (11βHSDs), types 1 and 2, have been extensively studied in the interconversion of cortisone and cortisol. These isozymes have also been shown to interconvert C11-oxy C19 steroids, implicating 11βHSD2 in the progression and development of castration resistant prostate cancer (CRPC). We recently reported that the 11βHSD isoforms catalyse the interconversion of C11-oxy C21 steroids and subsequent to downstream metabolism contribute to C11-oxy C19 steroid production. This study determined the kinetic parameters, Km and Vmax, of these isozymes towards 11β-hydroxyandrostenedione, 11β-hydroxytestosterone, 11β-hydroxyprogesterone (11βOHP4), 21-desoxycortisol, 11-ketoandrostenedione, 11-ketotestosterone, 21-desoxycortisone, and 11-ketoprogesterone. Data showed that 11βHSD2 exhibits greater substrate turnover than 11βHSD1, suggesting that the 11βHSD2 reaction would occur more readily, demonstrating potential regulatory roles of 11βHSDs regarding the C11-oxy steroids. Furthermore, data indicated competitive inhibition of 11βHSD2 by 11βOHP4. In vitro metabolic studies showed that 11βHSD and 17α-hydroxylase are key in the conversion of C11-oxy C21 steroids to C11-oxy C19 steroids, providing evidence for their contribution to the pathophysiological environment of various diseases and disorders, such as CRPC, 21-hydroxylase deficiency, polycystic ovary syndrome and renal disease. This study reports, for the first time, the kinetic parameters of 11βHSD1 and 11βHSD2 towards these C11-oxy steroids and demonstrates the metabolism of C11-oxy C21 steroids to C11-oxy C19 steroids. Data suggests that 11βHSD isoforms play more complex roles than those previously assigned and may potentially contribute indirectly to diseases and disorders.
Computational model of steroid biosynthesis via the delta-4 and delta-5 pathways

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The levels of specific steroid hormones in the body can have an effect on certain diseases such as heart disease and hormone-sensitive cancers. The enzymes of the delta-4 and delta-5 pathways have a big influence on the production of different steroid hormones. These enzymes determine the ratio of the synthesis of different steroids as well as the flux through the different branches of the pathways. The enzymes use multiple substrates and some of these substrates are shared between enzymes. The interlinked delta-4 and delta-5 pathways also contribute to the difficulty of monitoring the synthesis of the steroids when the enzyme activities are varied. There are few models that describe the complex kinetic reactions of steroidogenesis properly. Existing models that characterise the delta-4 and delta-5 pathways have limitations and lack accompanying experimental data. An accurately parameterised model of the pathway in humans will be beneficial for drug development, identifying possible drug targets as well as understanding diseases influenced by imbalances in the flux through the pathways. We are constructing an improved model for androstenedione synthesis via the delta-4 and delta-5 pathways and parameterising it for the ovine species. Parameter values are determined with the use of experimental in vitro data, depicting realistic saturation kinetics of the delta-4 and delta-5 pathways in sheep. An identifiability analysis of all newly determined parameter values is conducted. Much focus is placed on the mathematical methods behind data analysis and the importance of identifiability analysis of newly parameterised models. The improved model for the delta-4 and delta-5 pathways in sheep highlights the importance of thorough statistical analysis in model development. It is shown how steroidogenic models can be improved with the application of identifiability analysis. In future these methods will be applied to the human model (and other species) for improvement thereof.
Steroids are found predominantly sulfated in the human circulation. A group of steroid sulfotransferases makes these sulfate-adducts, mainly in the adrenal, the gonads and the liver, to expedite circulatory transit. This not only facilitates renal excretion, but also fuels peripheral de-sulfation and downstream steroid conversion and signaling. These human sulfotransferases need active sulfate in the form of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) and PAPS is exclusively produced by two enzymes, PAPS synthases 1 and 2. Mutations in the gene for PAPSS1 have never been clinically reported so far. Patients with inactivating mutations in PAPSS2, however, present with different degrees of bone and cartilage deformities as well as a specific steroid sulfation defect. The androgen precursor dehydroepiandrosterone (DHEA) fails to be converted to its sulfate ester DHEAS and downstream conversion leads to androgen excess and metabolic disease. This apparent deficiency in the DHEA sulfotransferase SULT2A1 could not be compensated by the intact PAPSS1 gene. Our latest study sheds light on this phenomenon: it seems that PAPSS2 and SULT2A1 interact functionally and physically with each other for streamlined steroid sulfation.

Steroid Metabolomics: A Powerful Technique for Differentiating Inborn Disorders of Steroidogenesis

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Background: Urinary steroid profiling by Gas Chromatography–Mass Spectrometry (GC-MS) provides a wealth of information regarding an individual’s adrenal function and is therefore considered the biochemical reference standard for diagnosis of Congenital Adrenal Hyperplasia and Disorders of Sex Development. Using ratios of steroid metabolites to assess specific enzymatic function has enabled analysis of profiles from single random urine samples as well as 24hour urine collections, making this more feasible for clinical use. Determination of normative limits for these ratios is challenging as the steroid metabolome changes throughout life and interpretation requires considerable expertise. Methods: We performed multi-steroid profiling by GC-MS in urine samples from 829 healthy controls and 192 patients with inborn steroidogenic disorders. This included genetically confirmed deficiencies in the following enzymes: CYP21A2 (n=27), CYP11B1 (n=13), CYP17A1 (n=30), POR (n=37), HSD3B2 (n=22), SRD5A2 (n=51), HSD17B3 (n=8) and in the co-factor cytochrome b5 (CYB5A; n=4). We assessed the diagnostic performance of 14 steroid precursor-to-product ratios, previously proposed as indicative for distinct steroid disorders. We compared this to the performance of a machine learning based steroid metabolomics algorithm. This involves analysis of GC-MS acquired profiles by a custom-designed approach, Angle Learning Vector Quantization (ALVQ), which classifies samples by comparing similarity (quantified as cosine of the angle) of their steroid metabolome to representative steroid metabolome prototypes for each enzyme deficiency. Results: Analysing the performance of the 14 established ratios, we found acceptable sensitivity and specificity. However, the automated steroid metabolomics approach performed significantly superior to this, in particular with regard to specificity. Conclusion: We present a novel steroid metabolomics approach, able to automatically diagnose inborn steroidogenic disorders, with improved performance when compared to the current reference standard. Steroid metabolomics is able to expedite and standardise interpretation of complex urinary steroid metabolome data and has excellent potential for implementation into routine clinical practice.
**Objectives:** Oestrogen analysis using liquid chromatography mass spectrometry is problematic, as oestrogens do not readily ionise. This coupled with low concentrations in men, pre-pubertal and post-menopausal women provides an analytical challenge. We investigated N-Methyl Pyridine-3-sulfonyl chloride (NMPS) derivatisation, as described by Wang et al (Steroids 2015 Apr;96:140-152) to improve sensitivity of 11 oestrogens; oestrone (E1), oestradiol (E2), 2-hydroxy-oestrone, 4-hydroxy-oestrone, 16-hydroxy-oestrone (oestriol-E3), 2-methoxy-oestradiol, 2-hydroxy-oestradiol, 4-hydroxy-oestradiol, 2-methoxy-oestrone, 11β-hydroxy-oestradiol. **Methods:** NMPS derivatisation is a two-step process. A pyridine sulfonyl group is first added to hydroxyl groups on the aromatic ring, then treatment with iodomethane adds the N-methyl group, the new molecule termed an oestrogen-NMPS. We used a Waters Xevo-XS with Acquity UPLC, a HSS T3, 1.8µm, 1.2x50mm column with water and methanol (both with 0.1% formic acid) as elution solvents over five minutes. **Results:** Six of the non-derivatised oestrogens were chromatographically separated. Both the 2- and 4-hydroxy metabolites of E1 and E2 co-eluted. LOQ ranged from 0.05 to 0.2ng/mL. Following NMPS derivatisation sensitivity for most analytes at least doubled with LOQ ranging from 0.025 to 0.2ng/mL. Again, six of the 11 oestrogens chromatographically separated. However, both single and double derivatised products of the 2 and 4-hydroxy oestrogens were observed, adding to the complexity of the method. Excluding these analytes the method was reproducible with repeatability measured as relative standard deviation of less than 10%. Matrix effects were less than ±20%, process efficiency and absolute recovery were between 30 and 50%. Further optimisation of the derivatisation procedure is required to improve recovery and to produce only the double derivatives. **Conclusions:** NMPS derivatisation improves oestrogen sensitivity in mass spectrometry, and could provide the sensitivity required for low concentration oestrogen analysis in numerous conditions.
Insulin resistance is the underlying cause of type II diabetes, a key risk factor for heart disease and other health issues. Glucocorticoids (GCs), steroid hormones known for their anti-inflammatory action, are known to induce insulin resistance. Similarly, an increase in pro-inflammatory cytokines has also been associated with the inability of cells to respond to insulin. This is curious seeing as GCs are anti-inflammatory and are well known to antagonize the effects of the pro-inflammatory cytokines. However, not much research has been done on the effects of the co-regulation by the GCs and the pro-inflammatory cytokines on the insulin signalling pathway. We have studied the effect that treatment with dexamethasone, a synthetic GC, followed by co-treatment with either tumour necrosis factor alpha (TNF-α) or interleukin-6 (IL-6), well-documented pro-inflammatory cytokines, has on key proteins in the insulin signalling pathway in mouse and human hepatocytes. Furthermore, we look at the effect of this co-treatment on gluconeogenesis by studying the mRNA expression on two key gluconeogenic genes, G6Pase and PEPCK, in addition to the key enzyme in glycogen synthesis, glycogen synthase. We report that while both dexamethasone and TNF-α inhibit insulin receptor protein expression, a more pronounced decrease is observed when liver cells are co-treated with both signalling molecules. Similarly, insulin receptor mRNA expression was also inhibited in the presence of both dexamethasone and TNF-α. Additionally, we demonstrate that co-treatment of dexamethasone with TNF-α affect downstream intermediates in the insulin-signalling pathway, such as insulin receptor substrate and AKT (Protein Kinase B) protein expression and activation. These results suggest that although GCs and pro-inflammatory mediators are known to have opposing biological effects this does not necessarily hold true in all instances. Furthermore, this study highlights a more integrated role for both stress and inflammation in promoting an insulin resistant state.
Comparison of the serum levels of androgens, precursors and metabolites in postmenopausal women and men: Identification of the major role of DHEA as source of androgens in the human

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Objective: To profile ten circulating unconjugated androgen-related steroids including 11 oxygenated compounds in sera from men and women in order to identify the mechanisms of androgen production and metabolism in the human. Methods: Ten androgen-related steroids have been measured in sera from postmenopausal women aged 51-68 years (n=44) and men aged 50-69 years (n=36) using validated LC MS/MS methodology. Results: In the same age range, men and postmenopausal women have serum steroid levels measured at the following respective concentrations for androstenedione (487±38.5 vs 333±22.2 pg/mL, mean ± SEM), 11β-hydroxyandrostenedione (1486±118 vs 1230±127 pg/mL), 11-ketoandrostenedione (283±19.5 vs 210±17.4 pg/mL), 11-ketotestosterone (328±36.1 vs 244±19.5 pg/mL) and 11β-hydroxytestosterone (139±14.6 vs 109±11.9 pg/mL) while the concentrations of testosterone and dihydrotestosterone in men are 19 times and 11 times higher, respectively, compared to postmenopausal women (4400±272 vs 227±20.3 pg/mL and 386±34.6 vs 34.9±2.1 pg/mL, respectively). In both groups, there was no detectable 11-ketodihydrotestosterone nor 11-hydroxydihydrotestosterone (less than or equal to 20 pg/mL), even in the serum with high concentrations of 11-ketotestosterone, for example, over 1000 pg/mL. Conclusion: In adult men, circulating testosterone(T) and dihydrotestosterone(DHT) originate essentially from the testes. The observation that the 11-oxygenated androgen-like steroids are present at somewhat comparable levels in both men and postmenopausal women of the same age indicates that these steroids diffuse into the circulation from the intracellular metabolism of DHEA of adrenal origin in the peripheral tissues under the control of intracrinology. The data further indicate a very low transformation of 11 KT to 11 KDHT and suggest that KT is directly eliminated by glucuronidation or sulfation without being transformed into DHT derivatives. The very low (<20 pg/mL) serum levels of 11K DHT thus indicate that KT is metabolised independently from DHT, thus avoiding significant androgenic stimulation in the prostate where DHT is the main active androgen.
Muscle stem cells, also known as satellite cells, are essential for muscle regeneration upon injury throughout life. Thus, the maintenance of quiescent muscle stem cells is crucial for maintaining muscle integrity. Serum levels of testosterone and estradiol gradually increase in mice after pubertal onset, and these levels are maintained during adulthood, suggesting that sex hormones may be implicated in the establishment and maintenance of muscle stem cells. We previously reported that the hypothalamus-pituitary-gonad axis establishes a reserve pool of adult muscle stem cells at puberty. However, sex steroids decrease in old age, which might result in functional decline of muscle stem cells. Here we found that administration of a Nal-Lys gonadotropin releasing-hormone antagonist (ante) to block the hypothalamus-pituitary gland-gonad axis for 4 months resulted in premature ageing of muscle stem cells. Although body weight and muscle mass were not changed, muscle regeneration upon BaCl2-injury was markedly reduced by ante treatment. Consistently, senescence-associated beta-galactosidase activity, p15, p16Ink4a, and rH2AX were increased in ante-treated MSCs compared to those of controls, whereas the expression of LaminB1 decreased. Intriguingly, functional decline of aged MSCs was completely rejuvenated by administration of dihydrotestosterone and 17beta-estradiol. We will discuss how sex steroid hormones are implicated in the cellular senescence of muscle stem cells.
Differential in vitro metabolism of clinically-relevant progestins used in contraception

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Steroid hormones regulate a variety of physiological processes including immune responses and reproductive function. A variety of synthetic progestogens designed to mimic the actions of the endogenous progestogen, progesterone (P4), are widely used for contraception and hormonal replacement therapy in women. While injectable progestin-only contraceptives containing medroxyprogesterone acetate (MPA) or norethisterone (NET) are commonly used in the developing world, other progestins such as levonorgestrel (LNG), etonogestrel (ETG) and nestorone (NES) are being explored for contraceptive use intravaginally or in multipurpose prevention technologies. Results from clinical trials and epidemiological studies suggest that the use of some progestins cause several side-effects, including increased risk of breast cancer, cardiovascular risk and most likely increased susceptibility to genital tract infections. The relative side-effects and molecular mechanisms thereof for different progestins are not well understood, while the contribution of metabolism is largely unexplored. We developed and validated an ultra-high-performance supercritical fluid chromatography-tandem mass spectrometry (UHPSFC-MS/MS) assay for quantifying the metabolism of these seven steroids simultaneously within several human cell lines and tissue from the female genital tract, and breast cancer cell lines. We show that MPA, NET, LNG, ETG and NES, as well as P4 and the synthetic glucocorticoid receptor agonist dexamethasone, are differentially metabolised in a ligand- and cell-specific manner. Considerable metabolism of P4 was observed in all cell lines; hence, a high-resolution screening method was employed to identify possible metabolites. A single major metabolite, 20(S)-hydroxyprogesterone, was identified in some of these cell lines and in the tissue, suggesting physiological relevance. This work contributes to the basic understanding of cell-specific progestin metabolism and these findings have important implications for in vitro experiments utilising P4 treatment. Furthermore, the results have potential relevance for side-effects for women on contraception or HRT, and choice of progestin.
Acute phase proteins induces an insulin resistant state

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Insulin resistance is the main risk factor for type-2 diabetes (T2D). The pathophysiology of insulin resistance is poorly understood and involves the insulin signalling pathway, which is activated by insulin, eliciting a biological response in target tissues via key proteins such as the insulin receptor (IR), insulin receptor substrates (IRS) and protein kinase B (Akt). Interestingly, the induction of insulin resistance is closely associated with chronic inflammation and stress and is characterized by increased levels of acute phase proteins (APP’s). Therefore these proteins are routinely used as biological markers for T2D, yet little is known about their effect on insulin signalling. We investigated the effect of treatment with APP’s such as plasminogen activator-1 (PAI-1), a coagulation marker, serum amyloid A (SAA) and C-reactive protein (CRP), on the mRNA and protein expression, and activation of key mediators of the insulin signalling pathway in liver cell models. Additionally, we investigate the effect of these APP’s on glucose uptake in target tissues (liver, muscle and adipose). We also show the effects of APP’s on glucose metabolism in liver cells, one of the outcomes of glucose uptake. Gluconeogenesis, which is inhibited by insulin in the liver, and glycogen synthesis, which is stimulated by insulin, was investigated by measuring the mRNA expression of two key gluconeogenic genes, G6Pase and PEPCK and the gene encoding for glycogen synthase, GYS. Having established that the acute phase proteins play a role in disrupting insulin signalling, we show that the APPs are upregulated in response to both tumour necrosis alpha (TNFα) and the synthetic glucocorticoid, dexamethasone. This suggest that GC-and inflammation-induced insulin resistance might be due to an increase in acute phase protein expression. This study highlights the role of APP’s as more than just biological markers for T2D, but key role players in the development of insulin resistance.
The phytoestrogen genistein exerts antioxidant activity, modulates cell migration and angiogenesis-related genes expression leading to improved skin flap viability in rats

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Objective: Selective estrogen receptor modulators (SERMs) have been developed to achieve beneficial effects of estrogens while minimizing their side effects. In this context, we decided to evaluate the protective effect of genistein, a natural SERM, on skin flap viability in rats and in a series of in vitro experiments on endothelial cells.

Methods: Three months prior to the flap surgery, rats underwent either ovariectomy or were sham operated. Consecutively, proximally based over-dimensioned random pattern skin flaps measuring 2 × 8 cm were dissected free from the underlying fascia on the back of each animal. Untreated groups received daily intramuscular injection of saline, while treated animals were daily administered with 1 mg/kg of genistein. The flaps viability was evaluated macroscopically and at the histological level. The in vitro study was conducted on HMVEC and HUVEC cells. Here, viability, migration, gene expression profiling and anti-oxidative properties were evaluated in endothelial cells following genistein treatment.

Results: Our results showed that administration of genistein increased skin flap viability, but importantly, the difference is only significant when treatment is started 3 days prior the flap surgery. Based on our in vitro experiments, it may be hypothesized that the underlying mechanism may rather be mediated by increasing SOD activity and Bcl-2 expression. The gene expression profiling further revealed 9 up-regulated genes (angiogenesis/inflammation promoting: CTGF, CXCL5, IL-6, ITGB3, MMP-14, and VEGF-A; angiogenesis inhibiting: COL18A1, TIMP-2, and TIMP-3). Conclusion: In conclusion, we observed a protective effect of genistein on skin flap viability which could be potentially applied in plastic surgery to women undergoing a reconstructive and/or plastic intervention. Nevertheless, further research is needed to explain the exact underlying mechanism and to find the optimal treatment protocol. Acknowledgement: Present study was supported by the Agency for Science and Research (under the contract Nos. APVV-16-0446, APVV-16-0207 and APVV-14-0731).
The testis is located in the scrotum outside the body cavity to maintain temperature 2–7°C cooler than the core body temperature. If the testis fails to descend into the scrotum during development, it is exposed to an elevated temperature and loses germ cells. Tight thermoregulation of the testis is essential in most mammals, but it remains unclear why male germ cell differentiation occurs properly only at a low temperature. Here, we determined germ cell apoptosis in mice under a mild heat stress. The results showed that a mild but prolonged heat exposure (37°C, 8h) induced in early pachytene spermatocytes. Since the survival of spermatocytes at the early pachytene stage is sensitive to testosterone, the apoptosis of spermatocytes were determined in mice treated with antide, an antagonist of luteinizing hormone. The antide treatment did not affect spermatogenesis in two months. Antide effects on the heat sensitivity of spermatocytes were not significant either. We now test the long term effects of antide on male germ cell development and sensitivity to heat stress.
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