Welcome to the "Steroids, Mass Spectrometry and Endocrinology – Past, Present and Future" Symposium



A Scientific Symposium and the Celebration of the Academic Career of Steroid Mass Spectrometry Pioneer Cedric Shackleton

University of Birmingham, 22nd – 23rd April 2022

















Welcome

Dear Symposium Guests, Colleagues and Friends,

We are delighted to welcome you to Birmingham for these exciting two days of cutting-edge science in the areas of steroid biosynthesis and metabolism, steroid analysis, and their innovative clinical applications.

We have prepared this symposium also to honour the occasion of the 80th birthday of the MASSter of steroid mass spectrometry, Professor Cedric H.L. Shackleton and we are delighted that we will have more than 100 guests from all over the world, many of whom have worked very closely with Cedric for many years, including several of his former PhD students and postdoctoral fellows and many key collaborators.

Cedric Shackleton's career spans more than five decades and includes the development of several ground-breaking approaches and techniques that have advanced the field in a major way. His work has been recognised on multiple occasions including the award of the 2010 Schroepfer medal for Steroid and Steroi Research by the American Oil Chemists Association.

Birmingham marks important corner stones in Cedric's life:

- Here in Birmingham, Cedric was born 80 years ago, evacuated as a Newborn from the Women's Hospital destroyed by a German air raid, spending his first few days on earth in the Cadbury's Chocolate Factory with many other Birmingham mothers and babies.
- Here in Birmingham, Cedric has been a great colleague and honorary professor for two decades, supporting generations of students and fellows in the Institute of Metabolism and Systems
 Research (and before the 2015 foundation of the IMSR, in the old Division of Medical Sciences), patiently teaching them the art of steroid analysis.
- Here in Birmingham, Cedric has had a crucial role in helping us set up the University of Birmingham's Steroid Metabolome Analysis Core (SMAC), driving us to ever new and higher standards of steroid analysis.
- Here in Birmingham, Cedric was made an Honorary Graduate of the University of Birmingham in 2016, delivering his commencement speech in the Great Hall, packed with graduates and their families.
- And here in Birmingham we will now enjoy a great symposium in his honour, fittingly again in the impressive environment of the Great Hall.

Many thanks to all members of the Local and Scientific Organising Committees listed below for helping to put this meeting together, particular thanks to my long-suffering, fabulous personal assistant Christine Lloyd, who made invaluable contributions to the organisation of this meeting.

We are looking forward to two exciting days - Welcome to Birmingham!

Professor Wiebke Arlt

William Withering Chair of Medicine

Director of the Institute of Metabolism and Systems Research (IMSR)

University of Birmingham, Birmingham, UK



Cedric Shackleton in several of his natural habitats

Local Organising Committee

Wiebke Arlt James Hawley Alessandro Prete Angela E. Taylor Christine Lloyd

Scientific Organising Committee

Wiebke Arlt, Birmingham, UK
Irina Bancos, Rochester, MN, USA
Tülay Guran, Istanbul, Turkey
James Hawley, Manchester, UK
Johannes (Hans) Hofland, Rotterdam, The Netherlands
Michael W. O'Reilly, Dublin, Ireland
Alessandro Prete, Birmingham, UK
Nicole Reisch, Munich, Germany
Karl-Heinz Storbeck, Stellenbosch, South Africa
Angela E. Taylor, Birmingham, UK
Dimitra A. Vassiliadi, Athens, Greece
Stefan A. Wudy, Giessen Germany

Presenters and Attendees of the Symposium will have the opportunity to submit original research and review manuscripts to be considered for publication in a **Special Issue of the Journal of Steroid Biochemistry and Molecular Biology (JSBMB)**, courtesy of its Editor-in-Chief, Jerzy Adamski.

Stefan A. Wudy (Giessen, Germany) will serve as Guest Editor for this Special Issue, supported by Wiebke Arlt (Birmingham, UK) and Michaela Hartmann (Giessen, Germany)

Programme

Friday 22nd April 2022

The Great Hall, Aston-Webb Building, University of Birmingham

12:30-13:30h Registration material pick-up, Networking Lunch and Welcome

13:30-15:00h Session 1: Steroid Analysis – Past and Present

Chairs: Wiebke Arlt, Birmingham, UK Jeremy Tomlinson, Oxford, UK

13:30-13:45h Paul Stewart, Leeds, UK Technology touching lives.

For many years I was the clinical translational front for innovative diagnostic steroid platforms pioneered by Cedric – drove new mechanisms of disease. Research that transformed not only my life, but many patients with inherited disorders of steroid metabolism. Set in motion a completely new paradigm in Endocrinology – changing emphasis from our obsession with circulating hormone levels to tissue specific metabolism – paracrinology and intracrinology.

And most importantly of all – found a close family friend!

13:45-14:00h Celso Gomez-Sanchez, Jackson, MS, USA **18-oxocortisol: a journey.**

18-oxocortisol is a hybrid steroid, the product of the action of the CYP17A1 and the CYP11B2 enzyme on 11-deoxycortisol. I will address aspects of the discovery, role of the steroid in primary aldosteronism and the issue of site of biosynthesis and clarification of the adrenal localization of enzymes that have been previously deemed to have very specific localizations within the adrenal.

14:00-14:15h Norman Taylor, London, UK

Getting them young: identifying inborn errors of steroid metabolism in newborns.

My first encounter with an inborn error of steroid metabolism in a newborn was the day I first met Cedric Shackleton for an informal interview. Since then, after the identification of many new steroids, the uncovering of several new disorders and analysis of urine from tens of thousands of patients, we have yet to exhaust this endeavour. The focus on the newborn has been a bedrock for all that came after.

14:15-14:30h Stefan Wudy, Giessen, Germany

From "Heinold's First and Last Chance Saloon" to Steroid Metabolomics: Memories of a former Shackleton Postdoc.

The analysis of steroids using mass spec has not always been as self-evident as it is today. I had the privilege to witness the earliest steps of steroid GC-MS and LC-MS in the Shackleton lab as a postdoc providing me an excellent basis for my career. Cedric not only proved himself an ingenious academic, teacher and researcher but also knew how to combine hard work and enjoying life in a unique way.

14:30-14:45h William Griffiths, Swansea, UK

Sterols, bile acids and Cedric.

In my presentation I will show how a paper from Cedric changed my career. I will introduce our methods for oxysterol analysis; present some data for oxysterol measurements in plasma and CSF; and describe ongoing work to build a sterol/oxysterol atlas of mouse brain using mass spectrometry imaging.

14:45-15:00 Karl Storbeck, Stellenbosch, South Africa

11-oxygenated sex steroids.

My talk on 11-oxygenated sex steroids will provide an overview of the biosynthesis and metabolism of 11-oxygenated androgens, including their aromatisation to 11-oxygenated oestrogens. I will present data on the activity of these previously overlooked sex steroids and will provide a summary of the implications to disease states, including polycystic ovarian syndrome and castration resistant prostate cancer.

15:00-15:30h Coffee & Networking

15:30-17:00h Session 2: Steroid Analysis – Present and Future

Chairs: **Angela E. Taylor**, Birmingham, UK **Jonathan W. Müller**, Birmingham, UK

15:30-15:45h Finlay MacKenzie, Birmingham, UK

The state of play of measuring steroids in hospital laboratories.

I will present the state of the art in the UK in 2022, which, depending on your own levels of perfection, will either shock or amaze you!

15:45-16:00h Brian Keevil, Manchester, UK

Everything Steroids and Saliva.

The talk will focus on the use of salivary testing in the investigation of adrenal insufficiency and also mild autonomous cortisol secretion. Data will also be presented on the measurement of 11-oxygenated steroids in saliva samples.

16:00-16:15h Oscar Pozo, Barcelona, Spain

Determination of the steroid profile by mass spectrometric methods: a Continuous Evolution towards the Direct Report of Intact Conjugates.

In this talk I will present the potential of the direct determination of steroid phase II metabolites by LC-MS/MS. After a very brief historical review of the determination of steroids by MS, I will show that the direct detection of phase II metabolites is the logical next step in that evolution. I will present the main achievements, challenges, advantages and drawbacks of that determination.

16:15-16:30h James Hawley, Manchester & Birmingham, UK **Implementing Urine Steroid Metabolomics in clinical practice.**

Here, I will be presenting an overview of our progress so far as we look to introduce a combined liquid chromatography-tandem mass spectrometry-machine learning (LC-MS/MS-ML) assay into routine practice within the NHS to help aid the differential diagnosis of adrenal incidentalomas. I will be discussing the challenges we have encountered and how we have tried to circumnavigate them.

16:30-16:45h Eder Zavala, Birmingham, UK Modelling determinants of diurnal steroid secretion.

The talk will focus on how mathematical and computational analysis techniques can help extract new features from dynamic hormone profiles, infer crosstalk points between endocrine activity and other physiological processes (e.g., inflammation), and build confidence around characterising hormonal rhythmicity through sub-cutaneous, ambulatory microdialysis in humans.

16:45-17:00h Ruth Andrew, Edinburgh, UK

Steroid mass spectrometry – an image of life beyond chromatography.

MS has provided significant insights into steroid metabolism and our ability to trace using stable isotopes has opened doors to studying dynamic and tissue specific roles for steroid hormones. Current approaches rely heavily on UHPLC- triple quadrupole instruments. Most recent steps in dissecting tissue turnover have led to the implementation of MS Imaging to visualise regional maps of steroid. This talk will overview the opportunities now available in the use of high-resolution MS analysis and ion mobility separations, which are required to realise the full potential of MS imaging.

18:00-20:00h Poster Session & Drinks Reception

Edgbaston Park Hotel, Pevsner Room

20:00h onwards Dinner Reception, Networking, Poster Prizes & Party

Edgbaston Park Hotel, Writer's Suite

Saturday 23rd April 2022

The Great Hall, Aston-Webb Building, University of Birmingham

9:30-11:00h Session 3: Steroids & CAH / Steroids & Inflammation

Chairs: **Dimitra A. Vassiliadi**, Athens, Greece **Stefan A. Wudy**, Giessen, Germany

9:30-9:45h Nicole Reisch, Munich, Germany

11-oxygenated androgens in congenital adrenal hyperplasia

The talk will summarise our current knowledge on biosynthesis of 11-oygenated androgens in patients with congenital adrenal hyperplasia and their relationship to adrenal and gonadal dysfunction in patients with the condition.

9:45-10:00h Tülay Guran, Istanbul, Turkey

Steroid profiling and clinical phenotyping in rare CAH variants.

Early diagnosis and appropriate treatment of rare forms of CAH are important for countries where consanguineous marriages are common. However, our knowledge of the effects of these rare diseases on the steroid metabolome is limited. With LC-MS-based steroid profiling and detailed phenotyping, large data collection on these rare diseases can be achieved. For this purpose, I will give examples from our own studies.

10:00-10:15h Elizabeth Baranowski, Birmingham, UK

Urine steroid metabolomics for the differential diagnosis of inborn disorders of steroidogenesis.

Inborn disorders of adrenal steroidogenesis result from genetic mutations in distinct enzymes, causing a block to hormone production and lead to the conditions Congenital Adrenal Hyperplasia (CAH) and Disordered Sex Development (DSD). Each enzyme deficiency is characterised by a distinct pattern of excessive and deficient excretion of individual steroid metabolites, enabling diagnosis with urinary steroid profiling, traditionally by gas chromatography-mass spectrometry (GC-MS). In my talk I will show how combining urinary steroid metabolome profiling with intrinsically interpretable machine learning methods can differentiate inborn disorders of steroidogenesis and support our understanding of this group of diseases.

10:15-10:30h Hans Hofland, Rotterdam, The Netherlands

Prostate cancer and androgen metabolism.

After treatment with androgen-deprivation therapy, advanced prostate cancer inevitably becomes castration resistant. Insights into the development of castration resistance can aid in improving the treatment options for individual patients. This talk will discuss the different steroid- and steroid receptor-dependent mechanisms contributing to castration resistance, that have emerged in the last few years and allow for more tailored approaches of prostate cancer treatment.

10:30-10:45h Angela Taylor, Birmingham, UK **The Steroid Response to Trauma.**

I will discuss the effect of trauma on steroidogenesis. In serum from male patients, we have shown altered steroid metabolism 9 minutes after trauma. Generally, there is an increase in steroidogenesis in the first hour after injury with levels falling after 24 hours, except for the androgens, testosterone and DHT, which decrease from the time of injury onwards. Glucocorticoid concentrations thereafter fluctuate depending on the severity of the injury and interventions needed during recovery. Androgen production remains low until 6-8 weeks after injury. Eight weeks following trauma steroidogenesis starts to represent that of pre-injury, with androgens and glucocorticoids returning to baseline levels.

10:45-11:00h Rowan Hardy, Birmingham, UK

How steroid metabolism shapes inflammatory disease.

In this presentation, Dr Hardy will present his latest data exploring the inflammatory regulation and function of autocrine corticosteroid and androgen metabolism in leukocytes shape disease progression in rheumatoid arthritis using RNAseq, LC-MS and luminex platforms.

11:00-11:30h Coffee & Networking

11:30-13:00h Session 4: Steroids & Metabolic Risk

Chairs: **Karl Storbeck**, Stellenbosch, South Africa **Jan Idkowiak**, Birmingham, UK

11:30-11:45h Jeremy Tomlinson, Oxford, UK

Glucocorticoid metabolism and metabolic dysfunction.

The talk with explore the role that altered glucocorticoid metabolism can have on metabolic phenotype, both in the context of normal physiology, but also in conditions of endogenous and exogenous glucocorticoid excess. I will focus in particular on the role of the 11β -hydroxysteroid dehydrogenases type 1 (11β -HSD1), that regenerates and amplifies glucocorticoid action, plotting the 'ups and downs' of selective 11β HSD1 inhibition as a metabolic therapy.

11:45-12:00h Irina Bancos, Rochester, MN, USA

Clinical impact of mild autonomous cortisol secretion (MACS).

My talk will focus on the epidemiology, clinical presentation, diagnosis, and health consequences of MACS. MACS is the most prevalent hormonal abnormality in patients with adrenal incidentaloma, seen in 30-50% of patients tested with the overnight dexamethasone suppression test. I will summarize the published evidence and the emerging data of the impact of MACS on cardiovascular risk factors and morbidity, bone health, frailty, and mortality.

12:00-12:15h Alessandro Prete, Birmingham, UK

Mild autonomous cortisol secretion: impact on steroid and global metabolism.

Mild autonomous cortisol secretion in benign adrenal tumours is increasingly recognised as a frequent and key contributor to cardiometabolic disease in adults. In my presentation I will show how mass spectrometry-based steroid and untargeted metabolomics can provide mechanistic insights into the metabolic consequences of cortisol excess.

12:15-12:30 Laura Wittemans, Oxford, UK

Sex steroids, genes and metabolic risk in women.

I recently started a Wellcome Fellowship on understanding the role of steroid hormone imbalances in the aetiology of cardio-metabolic and reproductive diseases using genetic, -omics and clinical data from large-scale cohort studies. I will give an overview of my first findings, and outline key work by others.

12:30-12:45h Michael O'Reilly, Dublin, Ireland

PCOS - the female andro-metabolic syndrome.

My talk will focus on the role played by androgens in mediating metabolic dysfunction in women with PCOS, which affects up to 10% of all women. Androgens are increasingly recognised as a driver of disturbances in energy metabolism in metabolic tissues in human and animal studies. During my PhD and early postdoctoral years my interest was predominantly on action and metabolism of classic androgens in adipose tissue, and my focus has recently shifted to skeletal muscle and the 11-oxygenated androgen subclass.

12:45-13:00h Lina Schiffer, Cleveland, OH, USA

Classic and 11-oxygenated androgen activation in peripheral tissues.

The local activation of circulating androgen precursors plays a key role for the regulation of androgen action in peripheral tissues. In this talk, I will present the work from my postdoc time in Birmingham characterising the differential activation of classic and 11-oxygenated androgen precursors in human adipose tissue and blood cells with a focus on the role of aldo-keto-reductase 1C3.

13:00-14:00h

Networking Lunch & Farewell

Our Speakers and Session Chairs



Ruth Andrew Ruth.Andrew@ed.ac.uk is a Professor of Pharmaceutical Endocrinology in the University of Edinburgh, UK. She is also the Director of Edinburgh's Clinical Research Facility Mass Spectrometry core which specialises in small molecule analysis. Her research focus is glucocorticoid metabolism and cardiometabolic disease and how innovative MS approaches can help us understand the interplay between the two.

"Most of the methodology my team have developed today for steroid analysis sits on the shoulders of Cedric's pioneering work. We have strong links and friendships through the Society for Endocrinology with colleagues past and present in IMSR."



Wiebke Arlt w.arlt@bham.ac.uk is the William Withering Chair of Medicine at the University of Birmingham, UK, where she serves as the Director of the Institute of Metabolism and Systems Research (IMSR). She is also a Consultant Endocrinologist at the Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust. Her research investigates the role of steroid metabolism and action in health and disease, with a particular focus on adrenal endocrine disorders as well as the link between steroids and metabolic disease.

"I was introduced to Cedric by Paul Stewart and the late Ewa Malunowicz in 2003. We galvanised our collaboration by elucidating the biochemical and genetic basis of P450 oxidoreductase deficiency. Cedric has been instrumental in helping me build the University of Birmingham Steroid Metabolome Analysis Core. Through very regular visits to Birmingham, he has trained our mass spec team as well as numerous PhD students and postdoc, lending them a patient ear and providing them with lots of enthusiasm, sound support and expert advice."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=arlt-w%20shackleton-c&sort=pubdate



Irina Bancos bancos.irina@mayo.edu is Associate Professor of Medicine at Mayo Clinic, Rochester, Minnesota, USA, and works in the Pituitary-Adrenal-Gonadal subdivision of its Endocrinology division. She also serves as the Associate Program Director for the Endocrine Fellowship. Dr. Bancos' clinical and research interests include adrenal tumors, adrenal insufficiency, and Cushing syndrome. Dr. Bancos' career and passion for adrenal disorders was inspired heavily by the time she spent in Birmingham immersed in the dynamic and collaborative environment, and under guidance and

mentorship of Wiebke Arlt. Dr. Bancos currently holds several grants in the field of adrenal disorders.

"I was introduced to Cedric while in Birmingham UK (2012-2014), during a two-year research fellowship (Wiebke Arlt Lab) sponsored by the Mayo Foundation Scholarship. I recall that Cedric's name was mentioned during almost every meeting we had on steroid metabolomics. I remember pondering the impact one person can have on a team, directly and peripherally!"

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=bancos-i+shackleton-c&sort=pubdate



Elizabeth S. Baranowski <u>e.s.baranowski@bham.ac.uk</u> is a Paediatric Endocrinology Registrar currently working at Birmingham Children's Hospital. She undertook an MRC Clinical Research Training Fellowship at the IMSR, University of Birmingham, supervised by Wiebke Arlt, Peter Tino and Jon Deeks. Her research to date has focussed on the integration of urinary steroid profiling by mass spectrometry-based methods with statistical and computational analysis techniques in Congenital Adrenal Hyperplasia and Differences in Sex Development.

"Cedric has been an invaluable guide and mentor supporting me with this work. A source of seemingly endless knowledge with equivalent enthusiasm, patience and generosity to share it. I am fortunate that Cedric and I met regularly during my fellowship, allowing me to benefit from his wealth of knowledge on mass spectrometry, the intricacies of steroidogenesis in health and disease, and steroid metabolism."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=baranowski-es+shackleton-c&sort=pubdate



Celso Gomez-Sanchez <u>cgomez-sanchez@umc.edu</u> is Professor at the Department of Medicine of the University of Mississippi Medical Center in Jackson, Mississippi, USA.

"My research career started in 1971 studying primary aldosteronism, but in that decade we were interested in possible new mineralocorticoids to explain low renin hypertension with low levels of aldosterone. Extraction of gallons of urine from patients with low renin hypertension and from rats with adrenal regeneration hypertension led to the isolation of 19-nordeoxycorticosterone in the late 1970's.

We met Cedric in the late 1970's and collaborated in the identification of the precursors of the synthesis of 19-norDOC and published a paper together with their identification in 1982. This followed with several other collaborations and publications. We became interested in 18-oxocortisol and 18-hydroxycortisol in the early 1980's as a potential mineralocorticoid that could be important in primary aldosteronism and hypertension and have continued to work on their role. My presentation will address the site of synthesis of 18-oxocortisol in the human adrenal and a challenge of classical concepts of adrenal zonation in normal individuals and some patients with primary aldosteronism."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=gomez-sanchez-c+shackleton-c&sort=pubdate



William J. Griffiths W.J.Griffiths@swansea.ac.uk is Chair of Mass Spectrometry, Swansea University Medical School (there is a typo in the programme, it has me at Cardiff). Our research is centred on cholesterol biosynthesis and metabolism, particularly on oxysterols, the intermediates between cholesterol and bile acids and steroids.

"Both Cedric and I were students of Jan Sjövall in Stockholm (the greatest living expert on cholesterol metabolism), although during different decades. Our paths have crossed many times with similar interests in haemoglobin, nuclear receptors, steroids and Smith-Lemli-Opitz syndrome."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=griffiths-wj+shackleton-c&sort=pubdate



Tülay Guran <u>tulayguran@yahoo.com</u> works in Istanbul, Turkey, at the Marmara University Faculty of Medicine, Department of Pediatric Endocrinology and Diabetes, as a full-time clinical consultant and senior lecturer. The main focus of Tülay 's research has been to investigate the pathogenesis of adrenal insufficiency, inborn disorders of steroidogenesis and sex development (DSD).

"Between 2013 and 2015, I spent a stimulating research period as a postdoctoral EU Marie-Curie research fellow at the University of Birmingham,

advised by Wiebke Arlt and regularly interacting with Cedric Shackleton. My time in Birmingham was instrumental in helping me establish and progress the work of our steroid mass spectrometry facility at Marmara University."



Rowan S. Hardy <u>r.hardy@bham.ac.uk</u> is a Lecturer and Research Fellow at the University of Birmingham. His research aims to delineate the role of corticosteroid and androgen metabolism in driving the pathophysiology of chronic inflammatory diseases, such as rheumatoid arthritis. To achieve these objectives, Dr Hardy's lab specialise in inflammatory ex vivo human tissues and cell culture models, and murine transgenic models of disease.

"Throughout an array of studies, grants and papers, Cedric's expertise and insight of steroid metabolism have been instrumental in shaping the direction of my research. He has always made time to sit down with myself

and my students to talk through the technicalities of what we are examining and identify the correct way forward"



James Hawley james.hawley@mft.nhs.uk is a Principal Clinical Scientist based in the Biochemistry Department at Manchester NHS Foundation Trust but also undertaking a part-time PhD with the Institute of Metabolism and Systems Research at the University of Birmingham. To date, his research has largely focussed on targeted steroid hormone analysis and improving assay standardisation. However, with the PhD he is now moving into the world of larger steroid panels, metabolomics and machine learning.

"The first time I became familiar with the name 'Cedric Shackleton' was when I wrote a review article in 2016 on LC-MS/MS cortisol analysis. The paper was returned to me (quite rightly) by one of the reviewers with the comment 'the author has not mentioned any of the work by Cedric Shackleton'. I have since righted this wrong and become more familiar with Cedric's huge contribution to steroid mass spectrometry and have had the pleasure to meet him on a few occasion – every time I come away having learned something new."



Johannes (Hans) Hofland j.hofland@erasmusmc.nl is a Consultant Endocrinologist at the Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands. Dr. Hofland heads the ENETS Center of Excellence at the Erasmus MC Cancer Institute and is the chairman of the Dutch Belgian NeuroEndocrine Tumor Society. His clinical and research efforts focus on endocrine oncology, with particular interests in steroid oncology, neuroendocrine neoplasms and radionuclide therapy.

"During my Marie-Curie Intra-European Fellowship at the Arlt lab back in 2013-2014 Cedric took his time to teach me many different aspects of steroid biology and how to measure them through GC/MS and LC/MS-MS. It was a privilege to receive this one-on-one training."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/32711725/



Jan Idkowiak j.idkowiak@bham.ac.uk is a Associate Professor of Paediatric Endocrinology & Diabetes at the Institute of Metabolism and Systems Research (IMSR) at the University of Birmingham, UK, and a Consultant Paediatric Endocrinologist at the Birmingham Children's Hospital.

Jan loves steroids and the adrenal gland (partly due to Cedric). Jan's main research interest focuses on childhood androgen excess and how it is linked to metabolic dysfunction.

"I know Cedric since I started my PhD in Birmingham in 2009 and we have worked together to characterise the urine steroid metabolome in children and adults with rare inborn errors of steroidogenesis."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=idkowiak-j+shackleton-c&sort=pubdate

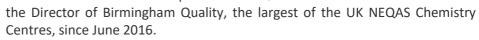


Brian G. Keevil <u>brian.keevil@mft.nhs.uk</u> I am a Consultant Clinical Scientist working in a routine NHS laboratory. Over the past 20 years I have developed a special interest in the investigation of steroid disorders using LC-MS/MS. My laboratory has built up a close working relationship with the CEDAM centre in Birmingham and we are helping them to develop a steroid panel for the investigation of adrenal cortical carcinoma.

"I first heard Cedric speak in the late 1970s at Northwick Park but I have only had the opportunity to work with him relatively recently."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/28422753/

Finlay MacKenzie <u>finlay.mackenzie@uhb.nhs.</u>uk is a Consultant Clinical Scientist at University Hospitals Birmingham NHS Foundation Trust. He is part of UK NEQAS since March 1987 and has been



While working in London, I was a junior collaborator in a project which was eventually written up as "The antenatal diagnosis and aid to the management of hereditary tyrosinaemia by use of a specific and sensitive GC-MS assay for succinylacetone", and for which I won £25 as a Poster Prize at an Association of Clinical Biochemistry meeting in Birmingham in 1983.

Almost 40 years later, I find myself as the Director of Birmingham Quality, part

of University Hospitals Birmingham, assessing the performance of hospital laboratories across the NHS and beyond. This includes, amongst a whole raft of chemistry analytes, the use of MS in Steroid measurement in clinical laboratories."



Jonathan W. Müller, <u>J.W.Mueller@bham.ac.uk</u></u> is Assistant Professor teaching Endocrine Biochemistry and Pharmacology at the University of Birmingham. Switch-like enzymatic reactions that steroid hormones are subjected to are his main research focus. Jon's research focus are dynamic sulfation and de-sulfation pathways of steroid hormones.

"I have joined the Birmingham steroid research group in 2012. From the very start, Cedric was there every now and then for the odd steroid-specific research question. We featured Cedric broadly at the SUPA 2017 conference here in Birmingham and in an associated editorial [1]. All the very best, Cedric!" [1] Mueller JW, Foster PA. Steroid sulfation research has come a long way. J Mol Endocrinol. 2018 Aug;61(2):E5-E6. doi: 10.1530/JME-18-0109. PMID: 30030389



Michael W. O'Reilly Michael.OReilly@rcsi.ie is a Clinical Associate Professor at the Royal College of Surgeons in Ireland (RCSI) and Consultant Endocrinologist at the Beaumont Hospital, Dublin, Ireland. His research is supported by a 4-year HRB Emerging Clinician Scientist Award, as well as the Wellcome Trust.

Mick's research focuses on the role of androgens in mediating metabolic dysfunction in women with PCOS, with a specific focus on the link between 11-oxygenated androgens and skeletal muscle energy metabolism.

He spent 8 years at the University of Birmingham from 2011 to 2019, completing his PhD funded by the Wellcome Trust in 2015 under the supervision of Wiebke Arlt, followed by a position as Clinician Scientist at the Institute of Metabolism and Systems Research (IMSR) from 2015 to 2019.

"During my time in Birmingham I was privileged to have access to Cedric's advice and mentorship and to his brilliant scientific mind. He was always available as a source of invaluable advice for all queries steroid- and mass-spec related."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/32711725/



Oscar Pozo opozo@imim.es leads the Research Group on Applied Metabolomics at the IMIM-Hospital del Mar Medical Research Institute in Barcelona, Spain. His research is focused on the direct determination of metabolites by mass spectrometric techniques. He has more than 15 years of experience in the determination and elucidation of steroid metabolites. In the last years, one of his main interests is the direct determination of steroid phase II metabolites.

"I discovered the conjugation of steroids with cysteine and I developed methods for the direct detection of poly-conjugates including bis-sulfates and sulfo-glucoconjugates. At that point, Cedric bumped into my research. We investigated the role of steroid poly-conjugates in prenatal diagnosis and we are currently exploring the potential of steroid poly-conjugates as potential biomarkers of adrenal cancer."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=pozo-o+shackleton-c&sort=pubdate



Alessandro Prete a.prete@bham.ac.uk is a Diabetes UK Sir George Alberti Research Fellow in the Institute of Metabolism and Systems Research at the University of Birmingham, UK, and an Honorary Consultant Endocrinologist at the Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust. His research focuses on functioning adrenal tumours, cortisol excess and novel therapies for adrenal insufficiency, combining clinical trials and experimental medicine studies.

"I have known Cedric since joining the University of Birmingham in 2016. He regularly provides advice on my work and research ideas, and I have been very fortunate in having access to his wealth of knowledge over the years."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=prete-a+shackleton-c&sort=pubdate



Nicole Reisch <u>nicole.reisch@med.uni-muenchen.de</u> is a Heisenberg Professor of Endocrinology at the Munich University Hospital, LMU Munich. Her clinical and scientific focus is on adrenal diseases and particular on congenital adrenal hyperplasia (CAH). She investigates the role and impact of androgen pathways in CAH and, in particular, their relevance as biomarkers for improving patient outcome. From 2008 to 2010 she worked as a EU Marie Curie postdoctoral fellow at the Institute of Metabolism and Systems Research (IMSR) at the University of Birmingham under the supervision of Wiebke Arlt.

"I got to know Cedric during my two years as a postdoctoral fellow in Wiebke's lab. There I dived into the steroid universe and I have been fascinated by it ever since. My postdoc period with Wiebke's group in Birmingham and all the discussions with Cedric over steroid profiles of pregnant women and newborns with P450 oxidoreductase deficiency were extremely stimulating and decisive for my career."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=reisch-n+shackleton-c&sort=pubdate



Lina Schiffer SCHIFFL2@ccf.org is a postdoctoral research fellow and has moved to the Cleveland Clinic Lerner Research Institute, Ohio, US, in early 2022 after spending five years in Wiebke Arlt's lab at the University of Birmingham. Her research is aimed at understanding, how steroid hormones are activated and inactivated in different tissues of the human body and how the dysregulation of these metabolic processes can contribute to the development of cancer and metabolic diseases.

"I met Cedric, when I was a postdoc in Birmingham (2016-2021) and in countless conversations Cedric shared his knowledge about steroids, mass spectrometry

and life with me. His enthusiasm for research is an inspiration to me and I am grateful to be able to pick his brain about all the crazy steroid metabolites I come across in my research."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=schiffer-l+shackleton-c&sort=pubdate



Paul M. Stewart p.m.stewart@leeds.ac.uk is Professor of Medicine, Adviser to the Vice Chancellor on Health Research and recently retired Dean at Leeds (and formerly Birmingham), He is a Consultant Endocrinologist and has a 30+year history of research focussing on the hypothalamo-pituitary-adrenal axis and specifically cortisol metabolism and its impact on human disease.

Through this, a 30+ year collaboration with Cedric Shackleton ensued that included funding Cedric's post at University of Birmingham and establishing the Steroid Mass Spectrometry facility in Birmingham that Wiebke and others working with Cedric have taken to a new level.

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=stewart-pm%20shackleton-c&sort=pubdate



Karl Storbeck storbeck@sun.ac.za is an Associate Professor in Biochemistry at Stellenbosch University, South Africa, and an Honorary Senior Research Fellow at the Institute of Metabolism and Systems Research (IMSR) at the University of Birmingham. Karl's research focuses on understanding the role of steroid hormone biosynthesis and metabolism in health and disease, with a special focus on investigating the metabolism and function of the overlooked adrenal 11-oxygenated androgens.

"I was first invited by Wiebke to visit Birmingham in 2014, and after identifying substantial synergy in our research interests I spent a six-month sabbatical in Birmingham in 2015. I was subsequently awarded a Newton Advanced Fellowship by the Academy of Medical Sciences,

which allowed me to make several visits to Birmingham during the period of 2016 -2019. It was during this time that I met Cedric who would regularly visit Birmingham. After a few visits we started synchronising our visits, and I had the privilege of leading a project to "download Cedric's brain" into two review articles about the steroid metabolome, which were published in 2019."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=storbeck-k+shackleton-c&sort=pubdate



Angela E. Taylor a.taylor.5@bham.ac.uk Angela has an analytical chemistry master's degree from Swansea University (2005). In 2009 she received her PhD from Swansea University investigating steroid metabolism in the human endometrium using mass spectrometry. This was followed by post-doctoral work at the University of Birmingham working for Prof Wiebke Arlt, which cumulated in setting up the SMAC facility in 2016. The SMAC lab conducts steroid research from wet lab based projects to clinical trials. Angela is interested in steroid metabolism in health and disease. Angela's role involves

the development of highly sensitivity, high throughput, well validated mass spectrometry assays for steroid analysis. Her research applies these assays to answer important clinical questions.

"I have been lucky enough to work with Cedric since 2009. Wiebke told me very early in my employment that it was my job to 'download' Cedric's brain, an impossible task for me alone, luckily we now have a team of people who are joining me with this mammoth mission, still a very long way to go. I admit to being apprehensive when I first met Cedric. Such a world renowned expert- I was nervous about all the things he'd ask me which I didn't know! However my apprehension was quickly abated when I met Cedric, he is so open and welcoming and his desire to share his knowledge with you is obvious. Cedric is most comfortable sitting with a coffee and drawing steroid structures, looking at mass spectra and sharing stories, or even happier doing the same but with a glass of wine in the evening. There aren't many people who can converse and engage with scientists of all ages and backgrounds in such an engaging way. It is a honour to work with such a lovely person, and long may we continue to discuss interesting data."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=taylor-ae+shackleton-c&sort=pubdate



Norman F. Taylor norman.taylor1@nhs.net is a former consultant clinical biochemist at King's College Hospital, having run a urine steroid profiling service there for 30 years and previously at Northwick Park Hospital for 13 years. The main research foci have been devising incremental improvements to the analytical method and reporting findings on the biochemistry of steroid disorders and their treatment. Our ascertainment of cases and the molecular expertise of Prof Arlt's group have combined fruitfully on several occasions.

"My first encounter with an inborn error of steroid metabolism in a newborn was the day I first met Cedric Shackleton for an informal interview. The new observations that I had witnessed that day were soon published. Since then, after the identification of many new steroids, the uncovering of several new disorders and analysis of urine from tens of thousands of patients, we have yet to exhaust this endeavour. The focus on the newborn in Cedric's own doctoral studies has been a bedrock for all that came after."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=taylor-n%20shackleton-c&sort=pubdate



Jeremy W. Tomlinson jeremy.tomlinson@ocdem.ox.ac.uk is Professor of Metabolic Endocrinology in the Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, UK. He spent his formative years in Birmingham (1999-2014), initially as a clinical research fellow learning the 'tricks of the trade' for a career in endocrinology and translational experimental medicine and subsequently as a clinical lecturer and eventually a Professor of Endocrinology.

"My first interactions with Cedric date back to my PhD and the clinical studies that we undertook looking at the impact of growth hormone as well as weight loss on global steroid metabolism and metabolic phenotype – we have continued to work together and collaborate ever since."

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=tomlinson-j+shackleton-c&sort=pubdate



Dimitra A. Vassiliadi dimitra.vas@googlemail.com I am currently working as a Consultant Endocrinologist in the Department of Endocrinology and Diabetes of Evangelismos Hospital, a major National Expertise Centre for Rare Endocrine Diseases and part of the Endo-ERN in the areas of adrenal, pituitary, and thyroid disorders. My research interests focus on the clinical and pathophysiological aspects of adrenal and pituitary disorders, especially in relation to all forms of Cushing's syndrome. An additional research interest relates to endocrine abnormalities in critical illness.

My special relationship with Birmingham started with the opportunity given to me by the Samuel Leonard Simpson Fellowship in Endocrinology from the Royal College of Physicians to work as a Clinical Research Fellow at the Department of Endocrinology, Division of Medical Science, University of Birmingham under the supervision of Professor Wiebke Arlt. Apart from the great scientific gain from this experience, I have very fond memories. I was not lucky enough to directly work with Professor Cedric Shackleton, but it was evident that his work was pivotal for all endocrinologists I met and had a special interest in the adrenals. Based on his huge impact on steroid biochemistry, I also was amongst those greatly inspired from his work that I very much value and respect. Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/32711725/



Laura Wittemans laura.wittemans@wrh.ox.ac.uk is a Sir Henry Wellcome Fellow and Postdoctoral Researcher at the University of Oxford hosted and mentored by Cecilia Lindgren. From 2014-2018, she undertook Wellcome Trustfunded PhD studies at the MRC Epidemiology Unit at the University of Cambridge advised by Claudia Langenberg, focussing on genetic approaches to identifying causal pathways to cardio-metabolic diseases. Prior to coming to the UK, she undertook a BSc in Bio-engineering at the University of Antwerp and an MSc in Bio-engineering at the University of Leuven, Belgium.

Laura investigates the biological mechanisms and consequences of steroid hormone dysregulation using genetics and functional genomics and Wiebke Arlt is one of the co-hosts for her fellowship. So far, she has participated in the Birmingham steroid research group meetings via zoom and looks forward to meeting Cedric and everybody else in person.



Stefan A. Wudy <u>Stefan.Wudy@paediat.med.uni-giessen.de</u> Stefan A. Wudy is a Pediatric Endocrinologist and Diabetologist with a strong scientific interest in steroid analysis as well as the metabolism of steroid hormones in health and disease. He learnt gas chromatography by Prof. Dr. J. Homoki (University of Ulm, Germany) who supervised his MD thesis. He received further training in analytical method development applying gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry as postdoctoral research fellow of the German Research Foundation (DFG) at the Steroid

Research & Mass Spectrometry Laboratory of Prof. Cedric H.L. Shackleton, Ph.D., D.Sc. at Children's Hospital Oakland Research Institute (California, USA).

Nowadays, Stefan is Professor of Pediatrics and Head of the Division of Pediatric Endocrinology & Diabetology at Justus Liebig University Giessen (Germany) and founded, along with his former doctoral student Michaela F. Hartmann Ph.D., an internationally renowned steroid laboratory offering worldwide service in mass spectrometry-based diagnostics of steroid related disorders as well as in steroid hormone research.

Joint work with Cedric https://pubmed.ncbi.nlm.nih.gov/?term=wudy-s+shackleton-c&sort=pubdate



Eder Zavala <u>e.zavala@bham.ac.uk</u> is a recently appointed Assistant Professor in Mathematics at the Centre for Systems Modelling & Quantitative Biomedicine at the University of Birmingham, UK. The SMQB is an interdisciplinary research centre spanning the Institute of Metabolism and Systems Research (IMSR) at the College of Medical and Dental Sciences and the Schools of Mathematics and Computer Science at the College of Engineering and Physical Sciences.

Eder's research explores mechanisms underpinning endocrine rhythms, particularly to answer the question of what makes them robust to some perturbations but fragile to others. He is also interested in developing non-

invasive ways to detect hormonal rhythm misalignment to support diagnosis.

"I joined the University of Birmingham in October 2019 and remember very well a very interesting pre-pandemic conversation with Cedric about the use of hormone ratios as biomarkers of abnormality, and how violin plots can better represent aggregated data on such biomarkers."

Poster Order

	Name	Title	Category
1	Marcel Schauermann, Giessen, Germany	Excretion of oxidated Cortisol Metabolites is markedly lower than previously assumed: An Analysis of Urinary Cortoic Acids in healthy Children by GC/MS	Steroid analysis
2	Bruno Lapauw, Ghent, Belgium	Free testosterone calculator inaccuracies in obese men	Steroid analysis
3	Daniyah Alamrani, Birmingham, UK	A liquid chromatography mass spectrometry method for the profiling of digoxin and other endogenous cardiotonic steroids in human serum	Steroid analysis
4	David Taylor, London, UK	Urine steroid profiling: an audit of test usage in a routine clinical laboratory in 2021	Steroid analysis
5	Fozia Shaheen, Birmingham, UK	Serum Multi-Steroid Profiling by UHPLC-MS/MS with Post- Column Infusion of Ammonium Fluoride	Steroid analysis
6	Joanne Adaway, Manchester, UK	Can we use Salivettes to collect samples for salivary androgen analysis?	Steroid analysis
7	Joeri Walravens, Ghent, Belgium	Binding Affinity Affecting SHBG SNPs Do Not Majorly Affect Calculated Estimates of Free Testosterone	Steroid analysis
8	Jonathan S Atkins, Manchester, UK	Serum cortisol immunoassay performance in the overnight dexamethasone suppression test	Steroid analysis
9	Laura Briggs, London, UK	Development and Validation of a High-sensitivity LC-MS/MS Method for Serum Oestrone and Oestradiol Quantitation without Derivatisation	Steroid analysis
10	Russell Brown, Glasgow, UK	Development and Validation of a Liquid Chromatography- Tandem Mass Spectrometry (LC-MS/MS) Method for the Analysis of Serum Testosterone and Dihydrotestosterone	Steroid analysis
11	Alan Griffiths, Stockport, UK	GCxGC-TOFMS as a complementary tool in doping control analysis of anabolic androgenic steroids	Steroid analysis
12	Miguel Debono, Sheffield, UK	Waking Salivary Cortisone as a Screening Test for Adrenal Insufficiency	Steroid biosynthesis
13	Hanna Nowotny, Munich, Germany	11-oxygenated C19 steroids are the predominant androgens responsible for hyperandrogenaemia in Cushing's disease	Steroid biosynthesis
14	Eylan Yutuc, Swansea, UK	Constructing three-dimensional sterol atlas of mouse brain	Steroid biosynthesis
15	Gido Snaterse, Ghent, Belgium	Dietary salt increases glucocorticoid exposure through modulation of downstream steroid metabolism and cortisol-binding globulin capacity	Steroid biosynthesis
16	Irundika Dias, Birmingham, UK	Altered mitochondrial oxysterol levels in Alzheimer's disease brain	Steroid biosynthesis
17	Mohsen Ali Asgari, Swansea, UK	Sterol and Oxysterol Markers of Huntington's Disease: The 24S- Hydroxycholesterol Pathway	Steroid biosynthesis
18	Oscar J. Pozo, Barcelona, Spain	Linkage between urinary phase II steroid profile in the course of menstrual cycle and mood factors	Steroid biosynthesis
19	Thomas Upton, Bristol, UK	The 24-hour dynamic relationship between tissue free cortisol and cortisone in healthy ambulatory volunteers	Steroid biosynthesis

20	Amarah Anthony, Birmingham, UK	Normothermic Machine Liver Perfusion as a tool to study human androgen metabolism	Steroid biosynthesis
21	Norman Taylor, London, UK	Cytochrome b5 deficiency: an XY disorder of sex development (DSD) that can now be detected by urinary steroid profiling at birth	Steroids and CAH
22	Lea Tschaidse, Munich, Germany	Improved biochemical control in salt-wasting CAH patients on stable fludrocortisone dose treated with modified-release hydrocortisone is associated with a fall in plasma renin activity and increase in sodium levels	Steroids and CAH
23	Clemens Kamrath, Giessen, Germany	Steroid metabotyping in treated infants with classical congenital adrenal hyperplasia by gas chromatography-mass spectrometry analysis	Steroid sand CAH
24	Fidéline Bonnet- Serrano, Paris, France	Decreased steroidogenic enzymes activity in benign adrenocortical tumors is more pronounced in bilateral lesions as determined by steroid profiling in LCMSMS during ACTH stimulation test	Steroids and adrenal tumours
25	Leah Braun, Munich, Germany	Gas chromatography-mass spectrometry urinary steroid metabotyping of patients with endogenous Cushing's syndrome	Steroids and adrenal tumours
26	Lorna Gilligan, Birmingham, UK	Urinary steroid metabolomics for adrenocortical cancer diagnosis: Comparison of gas chromatography-mass spectrometry to liquid chromatography-tandem mass spectrometry	Steroids and adrenal tumours
27	Prerna Dogra, Rochester, USA	Frailty in Patients with Adrenocortical Hormone Excess	Steroids and adrenal tumours
28	Catherine Zhang, Rochester, USA	Impaired Muscle Function and Health-Related Quality of Life in Patient with Mild Autonomous Cortisol Secretion	Steroids and adrenal tumours