Marco A. Rivarola and Alicia Belgorosky

Only one important subject, delivered at a Symposium during the meeting, will be herein commented. We believe that it touches upon a fundamental recent advance in the progress of medicine.

**SIMPOSIUM: STEM CELLS**

**IPS CELLS AS MODELS OF LIVER DISEASES: POSSIBLE THERAPEUTIC APPLICATIONS**

Gustavo Mostoslavsky

*Center for Regenerative Medicine, Boston University*

Reprogramming of somatic cells into embryonic-like pluripotent stem cells by overexpression of key transcription factors is a novel technology that promises to revolutionize human disease modeling and regenerative medicine. Induced pluripotent stem cells (iPSC) resemble human embryonic stem cells (hESC) in their morphology, growth and differentiation abilities. Both in vitro and in vivo they differentiate into mesoderm, endoderm and ectoderm lineages. iPSC have been already generated from individuals suffering from several human pathologies and their differentiation into relevant tissues allows for recapitulation of some of the disease features. The author’s laboratory is interested in endodermal-derived organ development, and more specifically in human iPSC in vivo differentiation into liver lineages and their potential application in disease modeling and therapy. They have previously developed a novel lentiviral vector for reprogramming of mouse and human somatic cells, based on a single polycistronic construct expressing all four reprogramming factors that allows for the most efficient reprogramming reported to date. Using this methodology, they have already generated more than a hundred normal and disease-specific human iPSC from patients suffering from two monogenic liver diseases, amyloidosis and hemochromatosis, and have shown their ability to differentiate into definitive endoderm followed by liver specification. The seminal work was published by Yamanaka’s group (Kioto University, Japan) who demonstrated the generation of iPSC from adult human dermal fibroblasts with four factors: Oct3/4, Sox2, Klf4, and c-Myc. Human iPSC were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity (Takahashi, Cell. 2007 Nov 30;131(5):861-72).

Using this approach they aimed to develop new methods to study the pathophysiologic mechanisms underlying these diseases, as well as, in the long-term, to correct their respective mutations using iPSC-derived liver cells for tissue regeneration. Since the source of iPSC is a tissue collected from the subject actually carrying the disease, two serious problems are simultaneously solved, first, there are no ethical and legal problems because the induced stem cells are derived from the patient rather than from an embryo or an umbilical cord, and second, immune incompatibility is bypassed. Finally, potentially, known gene defects could be potentially corrected in vitro by directed mutagenesis in the induced pluripotent stem cells. The source of stem cells might be a number of tissues. Hepatic tissue cells, skin fibroblasts, keratinocytes, and, more conveniently, peripheral blood cells have been used. Obviously, safety considerations, such as incomplete reprogramming and tumor genesis, need to be taken into consideration. The speaker also showed, as an example, that a complex organ, such as the rodent lung, can be engineered...
artificially from its interstitial and vascular framework, by providing it with lung iPSCs, to generate a functional parenchyma which was able to interchange CO$_2$ / O$_2$ as in normal respiration. **A new medicine is being born.**

See Figures 1-3

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**ILLUSTRATED CONCEPTS**

**Figure 1**

**Embryonic Stem cell (ESC) and adult Stem cell (SC)**

ESCs are totipotent or pluripotent in their capacity to differentiate into any cell of the body.

Most human ESCs are derived from embryos that develop from eggs that have been fertilized *in vitro* and then donated for research purposes with informed consent of the donors. They express specific transcription factors such as Nanog and Oct3/4. Transcription factors turn genes on and off at the right time. They can be grown and sub-cultured for many months.

Adult (somatic) stem cells are undifferentiated cells found among differentiated cells in a tissue or organ. They can renew themselves and differentiate to yield all of the major specialized cell types of that tissue or organ, but they are not pluripotent. They maintain and repair the tissue in which they are found.

**Figure 2**

**Induction of adult Pluripotent Stem cells (iPSC)**

**NUCLEAR REPROGRAMMING OF STEM CELLS**

Retroviral vectors → Replication → OKSM
Nucleus

<table>
<thead>
<tr>
<th>Adult stem cell (SC) in culture (fibroblasts, etc)</th>
<th>Induction</th>
<th>induced PSC (IPSC)</th>
</tr>
</thead>
</table>


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**SIMILARITIES BETWEEN EMBRYONIC STEM CELL (ESC) AND adult INDUCED PLURIPOTENT STEM CELLS (IPSC)**

- morphology
- surface antigens
- gene expression
- epigenetic status
- telomerase activity

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**Marco A. Rivarola and Alicia Belgorosky**

Servicio de Endocrinologia, Hospital de Pediatria Garrahan, Buenos Aires, Argentina

The general theme of Meeting was EVIDENCE-BASED PAEDIATRIC ENDOCRINOLOGY – ITS STRENGTHS AND LIMITATIONS. As stated by Chris Kelnar, ESPE 2011 President “A meeting of the highest scientific quality has been arranged that will give the opportunity in the Plenary Lectures, Symposia, Meet the Expert sessions and guided poster tours to look back at what we know scientifically, critically appraise the basis of that knowledge, showcase current research and look to the future as ESPE becomes an increasing international forum for the exchange of the latest scientific and clinical information”. To commemorate the 50th anniversary of ESPE, a copy of the new ESPE history book ‘ESPE – The First 50 Years’ edited by Wolfgang
Sippell, became available. It tells the story of ESPE’s development from a small club of friends into an international scientific society. Indeed, in Glasgow 1063 abstracts were submitted from 66 countries and 3200 delegates attended from 89 countries worldwide.

PLENARY LECTURES

PL1-1: Strengths and Limitations of Evidence-based Medicine.
The research community needs to serve the information needs of patients and professionals more effectively.
Sir Iain Chalmers
The James Lind Initiative, Editor, James Lind Library, Oxford, United Kingdom
Main points:
1. Good intentions are not enough to protect patients from unintended harm.
2. Systematic reviews of research should inform decisions in healthcare.
3. Biased under-reporting of research is unethical and should be outlawed.
4. We need less research, better research and research done for the right reasons.
5. New research should begin and end with systematic reviews of other relevant research.
Suggested action points:
1. Encourage admission of uncertainty about the effects of health practices and policies.
2. Increase the capacity for preparing, maintaining and disseminating systematic reviews of research evidence.
3. Outlaw biased under-reporting of health research and require public registration of controlled trials.
4. Promote research relevant to people planning, working in, and using the health services.
5. Refuse to support new research unless systematic reviews of existing evidence show that it is ethical and likely to be worthwhile.

We are transcribing a recent article published by the speaker on a related subject:
Use of research evidence in practice. Mark Petticrew, Iain Chalmers.
The Lancet, Volume 378, Issue 9804, Page 1696, 12 November 2011
We wish to comment on two aspects of Nancy Cartwright’s thoughts about the use of the results of research to guide decisions in policy and practice. First, we believe that to imply that this challenge can be addressed by using individual studies as the starting point is incorrect. Only very rarely will the results of a single trial provide a sufficiently reliable guide for policy or practice. Single trials (or other studies) addressing a particular question usually have implications for research, not practice. Where more than one similar trial is available, the appropriate starting point for assessing applicability in practice should be systematic reviews of all the relevant individual studies. Second, in trying to judge whether interventions studied in research “will work for us”, Cartwright, like many others, conceptualises the challenge as being to demonstrate that the characteristics and circumstances of the research are sufficiently similar to those to which extrapolation is being contemplated. But why should the challenge be conceptualised that way round? Why not instead ask “Are there any good reasons to believe that the research is not relevant to us, that ‘it won’t work for us’?” If there are not, and considering the undesirable alternative ways of reaching a decision, the default position should be that the result should be regarded as applicable. Fletcher has made a similar point in relation to subgroup analyses, suggesting that a good working assumption is that the main result probably applies to everyone, unless good evidence exists to the contrary.
We declare that we have no conflicts of interest.

References
1 Cartwright N. A philosopher's view of the long road from RCTs (randomized controlled studies) to effectiveness. Lancet 2011; 377: 1400-1401.
3 Fletcher J. Subgroup analyses: how to avoid being misled. BMJ 2007; 335: 96-97. London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK James Lind Initiative, Oxford, UK

Improving statistical quality in published research
Martin Bland
University of York, Health Sciences, York, United Kingdom.
Over the past 40 years, the quality of clinical research published in the major medical journals has improved greatly. I shall try to show how this has come about and identify key factors in this improvement. I shall go on to look at the position in the more specialised research literature and see what lessons can be drawn from this experience.

We are transcribing a recent article published by the speaker on a related subject:
Meta-analyses of adverse effects data derived from randomised controlled trials as compared to observational studies: methodological overview.
Golder S, Loke YK, Bland M. Centre for Reviews and Dissemination, University of York, York, United Kingdom. su.golder@york.ac.uk.
BACKGROUND: There is considerable debate as to the relative merits of using randomised controlled trial (RCT) data as opposed to observational data in systematic reviews of adverse effects. This meta-analysis of meta-analyses aimed to assess the level of agreement or disagreement in the estimates of harm derived from meta-analysis of RCTs as compared to meta-analysis of observational studies. METHODS AND FINDINGS: Searches were carried out in ten databases in addition to reference checking, contacting experts, citation searches, and hand-searching key journals, conference proceedings, and Web sites. Studies were included where a pooled relative measure of an adverse effect (odds ratio or risk ratio) from RCTs could be directly compared, using the ratio of odds ratios, with the pooled estimate for the same adverse effect arising from observational studies. Nineteen studies, yielding 58 meta-analyses, were identified for inclusion. The pooled ratio of odds ratios of RCTs compared to observational studies was estimated to be 1.03 (95% confidence interval 0.93-1.15). There was less discrepancy with larger studies. The symmetric funnel plot suggests that there is no consistent difference between risk estimates from meta-analysis of RCT data and those from meta-analysis of observational studies. In almost all instances, the estimates of harm from meta-analyses of the different study designs had 95% confidence intervals that overlapped (54/58, 93%). In terms of statistical significance, in nearly two-thirds (37/58, 64%), the results agreed (both studies showing a significant increase or significant decrease or both showing no significant difference). In only one meta-analysis about one adverse effect was there opposing statistical significance. CONCLUSIONS: Empirical evidence from this overview indicates that there is no difference on average in the risk estimate of adverse effects of an intervention derived from meta-analyses of RCTs and meta-analyses of observational studies. This suggests that systematic reviews of adverse effects should not be restricted to specific study types.
Is brown adipose tissue changing our metabolic world?

Barbara Cannon
Stockholm University, The Wenner-Gren Institute, Stockholm, Sweden

The presence of active brown adipose tissue in adult humans has been recognized in general physiology only since 2007. The intervening three years established that the depots originally observed by 18F-fluoro-deoxy-glucose positron emission tomography (FDG PET) scanning techniques really are brown adipose tissue depots because they are enriched for uncoupling protein 1 (UCP1). Reports of low apparent prevalence of brown adipose tissue based on retrospective studies of hospital records of FDG PET scans markedly underestimate true prevalence because such studies only reflect acute activity state; consequently, such retrospective studies cannot be conclusively analysed for factors influencing activity and amount of brown adipose tissue. Dedicated studies show that the true prevalence is 30-100%, depending on cohort. Warm temperature during the investigation — as well as adrenergic antagonists — inhibit tissue activity. There is probably no sexual dimorphism in the prevalence of brown adipose tissue. Outdoor temperature may affect the amount of brown adipose tissue, and the amount is negatively correlated with age and obesity. The presence of brown adipose tissue is associated with cold-induced nonshivering thermogenesis, and the tissue may be a major organ for glucose disposal. The decline in brown adipose tissue amount with increasing age may account for or aggravate middle-age obesity. Maintained activation of brown adipose tissue throughout life may thus protect against obesity and diabetes.

See Figures 4-6

ILLUSTRATED CONCEPTS

Figure 4

OBESITY/DIABETES CARDIOVASCULAR RISK

| Risk factors: High plasma levels of | Triglycerides (TG) | Cholesterol-rich remnants |

Figure 5

18F-FLUORO-DEOXY-GLUCOSE POSITRON EMISSION TOMOGRAPHY (FDG PET)

Activation of deoxy-glucose uptake

| Environmental Cold | Brown adipose tissue (BAT) imaging |

18F is a radioactive isotope of Fluor,
exhibiting an approximately 2 h half-life of type $\beta^+$ disintegration.

Figure 6

**BROWN ADIPOSE TISSUE (BAT) ACTIVITY CONTROLS TRIGLYCERIDE (TG) CLEARANCE.**
Bartelt A et al. (Nat Med 2011;17:200-5)

**ENVIRONMENTAL COLD INDUCTION**

Triglyceride-rich lipoproteins (TRLs) → TG → Lipoprotein lipase → TG → TG

CD36 Receptor → TG breakdown → UPC1

UPC1 = uncoupling protein 1. In BAT (but not white fat) mitochondria, and under catecholamine stimulation, it induces thermogenesis by uncoupling respiration from ATP production to dissipate energy.

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**PL2-4: Frontiers in Diabetes.**

**Immune therapy in type 1 diabetes mellitus**

Åke Lernmark

**Lund University/CRC, Clinical Sciences, Malmö, Sweden**

Background: The clinical onset of autoimmune (type 1) diabetes is occurring after a prodrome of varying length of islet autoimmunity. The warning signals for the ensuing loss of pancreatic islet beta cells are autoantibodies against insulin, GAD65, IA-2, and ZnT8, alone or in combinations. The prolonged subclinical islet autoimmunity prior to the clinical onset makes it possible to carry out controlled secondary prevention clinical trials of immune intervention to delay or prevent the clinical onset of the disease.

Objective and hypotheses: The objective is to screen newborn children for HLA genetic risk of type 1 diabetes. Islet autoantibodies are measured. The objective is to introduce immune modulating therapy in multiple autoantibody positive subjects to delay or prevent the clinical onset.

Methods: Autoantigen-specific immunotherapy with alum-formulated GAD65 (Diamyd®) will be compared to standard immune suppression with T or B lymphocyte monoclonal antibodies or other immunosuppressive agents.

Results: Immunosuppressive regimen has primarily been tested in intervention clinical trials. While Phase II clinical trials showed promising results, two Phase III trials have failed. Alum-formulated GAD65 show promise to reduce the loss of beta-cell function after the clinical onset of type 1 diabetes. The mechanisms are unclear but may involve the induction of T regulatory cells, which may suppress islet autoantigen reactivity. Past and on-going clinical trials have been safe. A prevention trial is underway.

Conclusions: Future clinical trials, perhaps as combination autoantigen-specific immunotherapy may increase the efficacy to prevent the clinical onset in subjects with islet autoantibodies or preserve residual beta-cell function in newly diagnosed type 1 diabetes patients.

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**PL4-5: ESPE Award Session and Activities 2.**
Effects of retraining eating speed on fasting and postprandial plasma ghrelin and PYY in obese children and adolescents
Julia Galhardo; Linda P Hunt; Stafford L Lightman; Matthew A Sabin; Cecilia Bergh; Per Sodersten; Julian Shield
1University of Bristol, School of Clinical Sciences, Bristol, United Kingdom; 2University of Bristol, Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, Bristol, United Kingdom; 3Royal Children's Hospital, Murdoch Children's Research Institute, Melbourne, Australia; 4Karolinska Institutet, Section of Applied Neuroendocrinology, Stockholm, Sweden

Background: Interactions between food consumption behaviour and gastrointestinal hormones have not been examined in detail in humans.

Objective: This study examined if retraining eating behavior aiming to slow down speed of meal consumption in obese adolescents was associated with changes in fasting and postprandial plasma levels of Ghrelin and PYY.

Methods: Twenty-seven obese adolescents participating in the Mandometer randomized controlled trial had plasma gut hormones measured in the fasting state and during an oral glucose tolerance test, at baseline and after 12 months of intervention. The Mandometer (a computerized scale), providing real time feedback during meals in order to slow down speed of eating was offered to 14 participants, whilst the control group (n=13) was treated with a diet and activity modification programme.

Results: Ghrelin and PYY responses at baseline in both groups were similar. At 12 months, those in the Mandometer arm exhibited greater absolute suppression of Ghrelin at 60 minutes (40.35 ± 2.37 pg/mL/min vs. 15.86 ± 6.17 pg/mL/min; p<0.001). PYY response at 90 minutes remained unaltered in the standard care arm regardless of weight loss attained (p=0.51), whilst all those in the Mandometer arm demonstrated a significant increase (p<0.001): at 12 months the estimated mean change in PYY between time 0 and 90 minutes in this group was 72pg/ml (95% CI 52-92 pg/mL). Change in meal duration correlated positively with change in absolute suppression in Ghrelin at 60 minutes (r = 0.515; p=0.006) and negatively with change in Ghrelin AUC (r = -0.487; p=0.009). These correlations persisted after controlling for change in BMI SDS.

Conclusions: Retraining obese adolescents to eat slower has a significant impact on the gastrointestinal hormone response to a carbohydrate load. We provide evidence that externally modifiable eating behaviours actually regulate the hormonal response to food rather than these hormones determining eating behavior.

PL4-6: ESPE Award Session and Activities 2.
MCM4 mutation is associated with adrenal failure, natural killer cell deficiency and increased chromosomal fragility
Claire Hughes; Eirini Meimaridou; Leonardo Guasti; Chen-hua Chuang; John Schimenti; Peter King; Colm Costigan; Adrian Clark; Louise Metherell
1WHRI, Bart's and the London School of Medicine, QMUL, Endocrinology, London, United Kingdom; 2Cornell University, College of Veterinary Medicine, Center for Vertebrate Genomics, Ithaca, United States; 3Our Lady's Children's Hospital, Crumlin, Paediatric Endocrinology, Dublin, Ireland

Background: A unique variant of Familial Glucocorticoid Deficiency exists in a genetically isolated population with high levels of consanguinity. Affected children develop hypocortisolaemia and raised ACTH but retain normal renin and aldosterone levels. Children also have short stature, evidence of increased chromosomal breakage and natural killer (NK) cell deficiency. Mutations in MC2R, MRAP and STAR were excluded.
Objective and hypotheses: Inheritance patterns were suggestive of autosomal recessive mechanisms therefore we tested the hypothesis that a single novel genetic disorder might underlie all major features including the adrenal failure.

Methods: We sought areas of homozygosity common to affected patients and subsequently interrogated these areas using massively parallel sequencing.

Results: Targeted exome sequencing identified one common variant (c.71-1insG) in mini chromosome maintenance complex component 4 (MCM4) that leads to aberrant splicing of exon 2 and is predicted to result in a severely truncated protein (p.Pro24ArgfsX4). Western blotting in patients revealed the absence of the major 96kDa isoform but a minor 80kDa isoform was retained. MCM KO in mice is lethal. Histological examination of the adrenals of MCM4Chaos3/- MCM3+/- mice, the closest viable animal model, revealed an abnormal adrenal morphology with increased apoptosis and reduced numbers of steroidogenic cells in the zona fasciculata, leading to lower corticosterone levels. This morphology is not seen in MCM4+/+ MCM3+/- mice.

Conclusions: MCM4 is one part of a heterohexameric complex essential for normal DNA replication and genome stability. MCM4 deficiency causes DNA replication defects and is a likely cause of the increased chromosomal fragility in these patients. It can also lead to cell apoptosis and we show higher levels of adrenal apoptosis in mutant versus wild-type mice. In summary we have identified the first human MCM4 mutation in a cohort of patients with a unique form of adrenal failure associated with short stature, NK deficiency and increased chromosome breakage.

See Figure 7

ILLUSTRATED CONCEPTS

Figure 7

FAMILIAL GLUCOCORTICOID DEFICIENCY
(High ACTH / Low cortisol levels)
neonatal hypoglycemic seizures

Loss-of-function mutations in the following genes

Type 1
ACTH receptor
(melanocortin 2 receptor, MC2R) 25%

Type 2
MC2R accessory protein (MRAP) 20%

Type 3
STAr protein 20%

New Type
MCM4

Melanocortin 2 receptor (MC2R): receptor for ACTH in adrenal cells
MC2R accessory protein (MRAP): trafficking of MC2R from ER to the cell surface and signaling (20%)
StAR protein: steroidogenic acute regulatory protein necessary for steroidogenesis
Mini chromosome maintenance complex component 4 (MCM4): it prevents adrenal apoptosis + other abnormalities.

PL5-7: New Paradigms in Molecular Medicine.
Exome analysis and the future of molecular medicine

Inês Barroso
The Wellcome Trust Sanger Institute, University of Cambridge Metabolic Research Labs, Inst. of Metabolic Science Addenbrooke's Hospital, Cambridge, United Kingdom

Family linkage studies, homozygosity mapping, candidate gene re-sequencing and genome-wide screening for chromosomal rearrangements were highly successful in identifying genes involved in monogenic/ Mendelian disorders. However, despite huge efforts, initial application of these approaches to complex traits were largely disappointing, with only a few of the putative associations holding up to replication. In 2007 the situation changed dramatically when the first genome-wide association studies (GWAS) were published and a number of loci reproducibly associating with human complex traits were identified. Since then more than 1200 loci associated with ~210 complex diseases and traits have been published (www.genome.gov/GWASStudies). Despite these successes, many Mendelian disorders remain with no known molecular cause, and for the most part the heritability of complex traits explained by common variants is small (<20% in aggregate for any given disease). Massively parallel sequencing approaches have opened the door to studies focused on sequencing all the protein coding exons (exome, less than 5% of the genome) in affected individuals in the hope of identifying causal genes. It has only been a couple of years since the proof of concept publication and more than 30 causative genes have been described using this approach. In my talk I will describe the use of this approach to identify causal genes implicated in Mendelian disorders as well as its extension to more complex diseases and traits. I will also highlight its likely impact on molecular medicine and clinical applications.

PL6-8: Food for Thought before Going Home.

The evolution of homeostasis and stress mechanisms

George Chrousos
University of Athens, First Department of Pediatrics, Athens, Greece.

Life maintains a complex dynamic equilibrium, homeostasis, challenged by intrinsic or extrinsic adverse forces, the stressors. Stress is the state of threatened homeostasis, that is re-established by a complex repertoire of physiologic and behavioral adaptive responses. Up to a certain threshold of a stressor strength and duration, the adaptive response reestablishes the usual healthy homeostasis or eustasis, without any cost to the individual. In contrast, when a stressor is not properly counteracted by the adaptive response and the homeostasis attained is suboptimal and associated with harm to the individual, this state is called allostasis or, more accurately, cacostasis. Finally, the organism may learn from a stressor and adapt and frequently attain and sustain a homeostasis that is better than would have been expected by its genetic and epigenetic constitution and the salient environment, i. e., a superior state, hyperstasis. The stress response is subserved by the stress system, located both in the central nervous system and periphery. The principal central effectors are highly interlinked, and include the hypothalamic CRH and arginine vasopressin, and the brainstem locus caeruleus and autonomic norepinephrine centers. The targets of these effectors are the brain, including the executive/cognitive, reward, and fear systems and the wake/sleep centers, the growth, thyroid and reproductive axes, as well as the gastrointestinal, cardiorespiratory, metabolic, and immune systems. Appropriate activity and responsiveness of the stress system is a prerequisite for a sense of well-being, successful performance of tasks, and positive social interactions. By contrast, inappropriate activity and responsiveness of this system may impair growth, development and body composition, and account for many neurobehavioral, endocrine, metabolic, cardiovascular, and allergic/autoimmune disorders. The genetic vulnerabilities of our species leading to the contemporary chronic
noncommunicable diseases resulted from selective pressures of evolutionary stressors upon our genome.

**PL6-9: Food for Thought before Going Home.**

**Growth promoting therapies in short children at the onset of puberty: an evidence-based appraisal – ESPE/JSPE**

*Toshiaki Tanaka*

Tanaka Growth Clinic, Pediatrics, Tokyo, Japan

It has been reported that among the clinical factors before early puberty, height at onset of puberty has the closest relation with adult height in patients with idiopathic short stature (ISS) or growth-hormone deficient short stature (GHD). Therefore, when children enter puberty at short stature, their adult stature remains short in both ISS without treatment and GHD even undergoing GH treatment. In Japanese boys, when the height at onset of puberty was above 135 cm, 88.9% exceeded 160 cm of adult height. But when the height at onset of puberty was below 135 cm, only 16.7% exceeded 160 cm. Combined GnRH analog and GH treatment was given to 26 short boys with GHD who entered puberty below 135 cm. 88% of GHD boys with combined treatment exceeded 160 cm of adult height, whereas 33% of GHD boys with only GH treatment reached above 160 cm. Mean adult heights in combined treatment group and in GH treatment group were 163.9 cm and 159.1 cm, respectively. Combined GnRH analog and anabolic steroid treatment was given to 21 short boys with idiopathic short stature who entered below 135 cm. 90.5% of ISS boys with combined treatment exceeded 160 cm of adult height. Mean adult height was 164.3 cm in combination treatment group and it was 156.9 cm in historical control group. Gonadal suppression therapy with GH or anabolic steroid significantly increased adult height in children who enter puberty at short stature.

**SYMPOSIA**

**EARLY LIFE ORIGINS OF HEALTH AND DISEASE**

**S2-13: Effect of in utero and early-life conditions on adult health and disease**

*Claire Levy-Marchal*

Hôpital Robert Debré, INSERM CIC EC 05, Paris, France

We are transcribing a recent article published by the speaker and colleagues on a related subject:


**Consequences of intrauterine growth and early neonatal catch-up growth.**

*Claris O, Beltrand J, Levy-Marchal C.* Department of Neonatology, Hospices Civils de Lyon, Lyon, France. [olivier.claris@chu-lyon.fr](mailto:olivier.claris@chu-lyon.fr)

The long-term consequences of small size at birth have been well described during the last 2 decades. It is important to assess the fetal growth velocity and to recognize that newborns may have growth restriction even with a normal birth weight. Intrauterine growth retardation suggests decreased growth velocity in the fetus as the result of a certain pathophysiologic process. An infant born after a short period of intrauterine growth retardation may not necessarily be small for gestation at birth. Several cohorts of adults born after a normal intrauterine growth have been followed for long term. Greater weight gain and fat mass early in life after thinness at birth are risk factors for overweight and cardiovascular diseases. Other risk factors include prematurity, bottle feeding, and tobacco exposure in utero. Early catch-up growth after fetal growth restriction replaces the organism on its growth trajectory with similar gain in weight and height.
S2-14: Epigenetic regulation of beta-cell function in fetal growth retardation
Rebecca Simmons
University Pennsylvania School of Medicine, Pediatrics, Philadelphia, United States.

Background: The abnormal intrauterine milieu of intrauterine growth retardation (IUGR) permanently alters gene expression and function of pancreatic b-cells leading to the development of diabetes in adulthood. Expression of the pancreatic homeobox transcription factor Pdx1 is permanently reduced in IUGR and epigenetic modifications are responsible for this decrease. Exendin-4 (Ex-4), a long-acting glucagon-like peptide 1 (GLP-1) analog, given on days 1-6 of life increases Pdx1 expression and prevents the development of diabetes in the IUGR rat.

Results: Here we show that Ex-4 increases USF-1 and PCAF association at the proximal promoter of Pdx1, thereby increasing histone acetyl transferase (HAT) activity leading to a permanent increase in histone H3 acetylation and H3K4 methylation. Normalization of these histone modifications precludes DNA methylation thereby preventing silencing of Pdx1 in islets of IUGR animals.

Conclusions: These studies demonstrate a novel mechanism whereby a short treatment course of Ex-4 in the newborn period prevents diabetes in adulthood by restoring Pdx1 promoter chromatin structure thus preserving Pdx1 transcription.

See Figures 8-11

ILLUSTRATED CONCEPTS

Figure 8
Intrauterine growth retardation (IUGR) has been linked to development of TYPE 2 DIABETES in the human adult

PDX1 GENE: pancreatic and duodenal homeobox 1 gene. The protein is a transcriptional factor activator of several genes; it is involved in the early development of the pancreas and plays a major role in glucose-dependent regulation of insulin gene expression postnataally.

Figure 9

EXPERIMENTAL IUGR

EPIGENETIC SILENCING OF THE PDX1 GENE

DEVELOPMENT OF DIABETES IN THE ADULT RAT

Authors (Park et al. J Clin Invest 2008;118:2316-24) found that expression of Pdx1 was permanently reduced in IUGR β cells and underwent epigenetic modifications throughout development:

DNA methylation  Pdx1 Transcription

PROXIMAL PROMOTER  Pdx1 GENE

Histone 3 deacetylation

IUGR induces permanent epigenetic changes

USP1: activator of transcription

Figure 10

Exendin-4 (Ex-4), a long-acting glucagon-like peptide 1 (GLP-1) analog, given on days 1-6 of life increases Pdx1 expression, reverses epigenetic changes and prevents the development of diabetes in the IUGR rat (Pinney S et al. Diabetologia, 201;54:2606-14):

DNA methylation  Pdx1 Transcription

PROXIMAL PROMOTER  Pdx1 GENE

Histone 3 acetylation

epigenetic changes

Ex-4 + PCAF Acetyl transferase

USF1

Figure 11
S2-15: Prenatal stress, glucocorticoids and the programming of adult disease

Jonathan Seckl
University of Edinburgh, Queen's Medical Research Institute, Edinburgh, United Kingdom.

Epidemiological evidence suggests that an adverse fetal environment permanently programmes physiology leading to increased risks of cardiometabolic, neuroendocrine and psychiatric disorders in adulthood. We originally hypothesised that fetal glucocorticoid overexposure might explain this link. In rodents, prenatal stress, glucocorticoid exposure or inhibition/knockout of 11b-hydroxysteroid dehydrogenase type 2 (11b-HSD2), the fetoplacental "barrier" to maternal glucocorticoids, reduces birth weight and causes permanent hypertension, hyperglycaemia, increased hypothalamic-pituitary-adrenal (HPA) axis activity and anxiety-related behaviours in adult offspring. The phenotype persists into a second generation and transmits via male and female lines. This implies epigenetic mediation, a mechanism emerging for HPA axis programming. This also appears of potential clinical relevance. Thus, in non-human primates, exposure to glucocorticoids in the second half of gestation programmes cardiometabolic, HPA and behavioural parameters in offspring. In humans, placental 11b-HSD2 activity correlates directly with birth weight and inversely with infant blood pressure. Moreover, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming. Indeed, maternal glucocorticoid therapy or ingestion of liquorice (which inhibits 11b-HSD2) alters offspring cognition, behaviour and HPA function. Stress has similar effects since pregnant women exposed to the 9.11.2001 atrocity and who developed PTSD "transmit" neuroendocrine changes to their one-year old offspring, but confined to third trimester exposure. Furthermore, exposure to the Nazi Holocaust exerted permanent effects upon glucocorticoid levels and steroid metabolism, effects dependent upon the age at exposure. The second (un-exposed) generation also shows altered cortisol levels and metabolism. Overall, the data suggest that developmental exposure to excess glucocorticoids/stress programmes functions in adult life and these effects may impact on a subsequent generation.

See Figure 12

**Figure 12**

**CORTISONE – CORTISOL SHUTTLE**

**IN PLACENTA**

**PHYSIOLOGY:**

<table>
<thead>
<tr>
<th>FETUS</th>
<th>PLACENTA</th>
<th>MOTHER</th>
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<tr>
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<td>11β-HSD1</td>
<td>11β-HSD2</td>
</tr>
<tr>
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<td>CORTISOL</td>
<td>CORTISOL</td>
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</table>

**PATHOLOGY:** Intrauterine growth retardation
THE CORTISOL-CORTISONE SHUTTLE IN HEALTH AND DISEASE

S8-31: Genetic syndromes caused by mutations in the 11β-hydroxysteroid dehydrogenases and implications for the HPA axis

Paul Stewart
University of Birmingham, Endocrinology, Birmingham, United Kingdom.

In mammalian tissues, two isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD) catalyze the interconversion of hormonally active cortisol (F) and inactive cortisone (E). 11β-HSD2 is a high affinity dehydrogenase expressed in adult kidney that inactivates F to E protecting the mineralocorticoid receptor (MR) (which has equal affinity for F and aldosterone in vitro) from cortisol excess. “Cushing’s disease of the kidney” occurs in the hypertensive condition “apparent mineralocorticoid excess (AME)” because of mutations in the HSD11B2 gene. Although peripheral conversion of F to E is severely impaired circulating F concentrations are normal because of HPA axis feedback and a concomitant reduction in daily F secretion rate.

By contrast, 11β-HSD1 is a bi-directional enzyme but in vivo the predominant action in liver, adipose tissue and muscle is E to F conversion where it augments glucocorticoid hormone action. The pivotal oxo-reductase activity of 11β-HSD1 is critically dependant upon the generation of NADPH within the endoplasmic reticulum from an accessory enzyme hexose-6-phosphate dehydrogenase (H6PDH). Mutations in the H6PDH gene explain the molecular basis for apparent cortisone reductase deficiency (ACRD) whereby patients present with hyperandrogenism, polycystic ovary syndrome phenotype and/or precocious pseudopuberty because of increased ACTH drive to the drive secondary to increased cortisol clearance. Recently we have described “true” cortisone reductase deficiency (CRD) as a milder phenotype of ACRD due to mutations in HSD11B1 itself. In parallel, recombinant mice lacking H6PDH and 11β-HSD1 have been used to evaluate the consequences upon HPA axis function. Mutations in the HSD11B2 and HSD11B1/H6PDH genes explain the monogenic diseases AME and (A)CRD and add insight into the regulation of HPA axis function in normal physiology and diseased states.

See Figures 13-14

Figure 13

CORTISONE – CORTISOL SHUTTLE

PHYSIOLOGY

H6PDH

ACTH
H6PDH: hexose-6-phosphate dehydrogenase: in liver, adipose tissue, and muscle.

11β-HSD1: 11β-hydroxysteroid dehydrogenase 1: in liver, adipose tissue, placenta and muscle.

11β-HSD2: 11β-hydroxysteroid dehydrogenase 2: in kidney and placenta

Figure 14

CORTISONE – CORTISOL SHUTTLE

PATHOLOGY. Monogenic diseases


11β-HSD2 gene deficiency: Apparent Mineralocorticoid Excess (AME)

IN RENAL TISSUE:
S8-32: 11beta-HSD1 inhibitors for metabolic syndrome and beyond
Brian R Walker
University of Edinburgh, Centre for Cardiovascular Science, Edinburgh, United Kingdom

Excessive activity of glucocorticoids causes obesity, type 2 diabetes, cardiovascular disease and cognitive impairment. The microsomal enzyme 11beta-HSD1 converts inert cortisone into active cortisol, thereby amplifying intra-cellular cortisol concentrations in liver and adipose tissue and increasing glucocorticoid receptor activation, independently of circulating cortisol concentrations. We proposed that inhibition of 11beta-HSD1 would reduce intracellular cortisol levels and hence glucocorticoid action without interfering with the normal stress response, providing a novel therapeutic approach in type 2 diabetes, obesity, and cognitive dysfunction. Proof of concept was confirmed in a series of mouse transgenic models and in humans using a prototype 11beta-HSD inhibitor, carbenoxolone. Moreover, we have used novel stable isotope tracers and other tools to quantify 11beta-HSD1 in vivo in humans, showing that activity is increased in adipose tissue in obesity and type 2 diabetes.

Selective inhibitors of 11beta-HSD1 have been developed in several pharmaceutical companies, and also by an in-house drug discovery team created within the University of Edinburgh. These show efficacy in a wide range of pre-clinical models, including models of cardiovascular lesion development, and encouraging efficacy in Phase II studies in type 2 diabetes.

S8-33: Adverse effects of maternal glucocorticoids – the role of the 11beta-HSD placental barrier
John R. Challis
Toronto, Canada.

We are transcribing a recent article published by the speaker and coworkers on a related subject:

Periconceptional undernutrition (UN) in sheep accelerates fetal hypothalamic-pituitary-adrenal (HPA) axis activation, resulting in preterm birth. In contrast, twin conception suppresses fetal HPA function and delays prepartum HPA activation. We hypothesized that these dissimilar effects on fetal HPA activity result from different influences of maternal glucocorticoid (GC) on maturation of the fetal HPA axis, mediated via different activities of placental 11beta-hydroxysteroid dehydrogenase (11betaHSD) isozymes. We examined the effects of twinning and maternal periconceptional UN from 60 days before until 30 days after mating on the ontogeny of placental 11betaHSD-1 and -2 enzyme activities. At day 85 of gestation, placental 11betaHSD-2 activity was lower in UN than in normally nourished (N) fetuses (P < .05) and was higher in twins than in singletons (P < .05). Furthermore, placental 11betaHSD-1 activity was not different between nutritional groups but was higher in twins than in singletons (P = .01). At day 85, fetal plasma cortisol (P < .001) and cortisone (P < .001) concentrations were lower in UN than in N fetuses, but the cortisol to cortisone ratio was higher in UN than in N fetuses (P = .01). There was no effect of fetus number on plasma cortisol or cortisone concentrations or on the ratio of cortisol to cortisone at day 85. Therefore, periconceptional UN and twinning may result in the alterations of placental 11betaHSD isozyme activities at
particular times during gestation. Changes in these activities during critical periods of fetal development could affect transplacental transfer or placental generation of GCs that reach the fetus, potentially influencing the timing of activation of the fetal HPA axis, fetal maturation, and hence the development and health later in life.

See Figure 12

NEW INSIGHTS IN THE PATHOGENESIS OF PCOS

S11-40: Transient in utero androgen excess and glucose intolerance: lessons for PCOS from animal models
David Abbott1; Lindsey Nicol2; Ning Xu3; Daniel Dumesic4; Mark Goodarzi5
1University of Wisconsin, Ob/Gyn and Wisconsin National Primate Res. Ctr., Madison, United States; 2Oregon Health Sciences University, Pediatrics, Portland, United States; 3Cedars-Sinai Medical Center, Medicine, Los Angeles, United States; 4University of California, Ob/Gyn, Los Angeles, United States; 5Cedars-Sinai Medical Center, Medicine and Ob/Gyn, Los Angeles, United States.
Fetal androgen excess induces polycystic ovary syndrome (PCOS)-like reproductive and metabolic traits in female mammals from rodents to primates. Recent studies in female rhesus monkeys demonstrate that maternal glucose intolerance is induced in tandem with androgen excess. Pancreatic accommodation of dual androgenic and glycemic gestational excess may contribute to adult metabolic pathophysiology in these prenatally androgenized (PA) female monkeys. To gain insight into potential molecular mechanisms involved in PA monkey pathophysiology, DNA methylation was examined in visceral fat from PA infant and adult females. Pathway and network analyses of differentially methylated genes implicated multiple signaling pathways. Transforming growth factor-beta (TGF-beta) signaling was amongst the top two pathways identified by gene methylation analyses in both PA infant and adult monkeys. As a potential gene candidate for PCOS, intron 55 of fibrillin 3, is involved in regulating TGF-beta signaling in women, altered function of this signaling pathway may accompany PCOS pathophysiology in fetal androgen excess animal models and in women.

S11-41: Searching for genetic factors in PCOS: new insights from China
Zi-Jiang Chen
Shandong Provincial Hospital, Shandong University, Obstetrics and Gynecology, Jinan, China

Background: Polycystic ovary syndrome (PCOS), a complex endocrine-metabolic disorder affecting 6-8% of child-bearing-aged women, is the most common cause of female anovulatory infertility. PCOS is associated with endocrine-metabolic derangements leading to a broad range of adverse sequelae that include dyslipidemia, atherosclerosis, insulin resistance, and type 2 diabetes (T2D). Despite many prior studies, genes responsible for PCOS have not been elucidated.

Methods: We performed a genome-wide association study (GWAS) of PCOS in Han Chinese from 21 provinces totaling 4082 PCOS patients and 6687 controls. The initial discovery set consisted of 744 PCOS patients and 895 controls, genotyped by Affymetrix SNP 6.0 chip. Associations were secondly interrogated in additional independent replications: 1) 2840 PCOS cases and 5012 controls from Northern China; 2) 498 PCOS cases and 780 controls from Central and Southern China.

Results: We identified strong evidence of associations between PCOS and 3 loci: 2p16.3 (rs13405728, Pmeta = 7.55c10-21, odds ratio 0.71); 2p21 (rs13429458, Pmeta = 1.73c10-23, odds ratio 0.67); and 9q33.3 (rs2479106, Pmeta = 8.12c10-19, odds ratio 1.34).
**Conclusions:** These findings provide new insight into the pathogenesis of PCOS, with follow-up studies of the candidate genes in these regions recommended.

**S11-42: Reproductive function in the offspring of women with PCOS: who is the chicken and who is the egg?**

_Fernando Cassorla_

University of Chile, Institute of Maternal and Child Research, Santiago, Chile

A significant body of knowledge has emerged during the last few years indicating that the offspring of women of PCOS may exhibit characteristic metabolic and reproductive features from a very early age, well before the onset of clinical and biochemical hyperandrogenism. It has been proposed that these features may represent early markers for the subsequent development of PCOS. In the case of daughters of women with PCOS, they may show evidence of increased serum anti- mullerian hormone (AMH) concentrations during the first few months of life, and these levels remain high during childhood and adolescence compared to controls. In addition, these girls may exhibit increased ovarian volumes, higher postprandial serum insulin concentrations and lower serum adiponectin levels compared to carefully matched controls, even before the onset of puberty. Furthermore, prepubertal daughters of women with PCOS may show evidence of an exaggerated adrenarche, with higher DHEAS responses to ACTH stimulation compared to controls. In addition to these findings, during pubertal development, the daughters of women with PCOS may show evidence of increased serum basal testosterone and post-stimulated LH, 17 OH progesterone and testosterone concentrations, at a time when some early clinical signs of hyperandrogenism may become evident. These features may be associated with higher serum levels of triglycerides, and lower concentrations of SHBG. In the case of sons of women with PCOS, they may also exhibit increased serum concentrations of AMH and higher BMIs, well before the onset of puberty. During adulthood, the sons of women with PCOS remain overweight, and show evidence of higher postprandial insulin concentrations and lower insulin sensitivity (adjusted for BMI), compared to controls.

**Conclusions:** The offspring of women with PCOS may show evidence of specific metabolic and reproductive features, several years before the onset of puberty. These features may place them at risk for the subsequent development of metabolic and reproductive derangements, including PCOS, during adulthood.

**ESPE Working Groups. Ovarian failure in Turner syndrome**

**WG5-76: Development of the ovary: genes and ovarian failure in TS**

_Luca Persani_

University of Milan and Istituto Auxologico Italiano, Division of Endocrine and Metabolic Diseases, Milan, Italy

Ovarian folliculogenesis is the fundamental process of ovarian development and function. Around 7 million primordial follicles are present in the developing ovary during embryogenesis. The large majority of these follicles are lost during fetal and postnatal life by atresia. In women with 45,X karyotype, oocyte loss occurs in the early stages of meiotic prophase, resulting in gonadal dysgenesis and primary amenorrhea. The Turner phenotype may be explained by several mechanisms but the most substantiated one is the haploinsufficiency of X-linked genes (such as SHOX) that physiologically escape X chromosome inactivation and are needed in two copies for ovarian function. The requirement for a double dosage of certain X-linked
genes is supported by the observation that complete spontaneous puberty can be reached in 30–40% of mosaic Turner patients. Characterization of partial X chromosome monosomies in women presenting the full Turner phenotype or only primary ovarian failure (POI) highlighted specific regions that might be involved in ovarian function. In addition, these studies seemed to favor haploinsufficiency for specific Xq or Xp genes as the cause of POI. All Xp deletions excluded most of the p arm and highlight the involvement of the proximal part of Xp in ovarian function. A locus was defined at Xp11.2–q22.1, within which mapped several of the Turner traits, including POI. Xq terminal deletions were relatively common and frequently very large. They were associated almost exclusively with secondary or primary amenorrhea and only rarely with other Turner traits. Larger deletions presented primary amenorrhea, whereas in deletions originating in Xq21 or further distally the more common phenotype was secondary amenorrhea. In women with normal fertility, a number of interstitial deletions in proximal Xq excluded most of the proximal part of Xq.

**WG5-77: Gonadal failure in Turner syndrome - prenatal, childhood, pubertal age**

*Theo Sas*
Rotterdam, Netherlands

Gonadal failure occurs in most individuals with Turner syndrome (TS). Germ cells in the genital ridges multiply normally into the millions by midgestation. Afterward, however, there is accelerated loss of oocytes by apoptosis in the 45,X ovary compared to the 46 XX ovary, leaving few follicles in a fibrous streak by birth in a girl with TS. Approximately one third of girls with TS have spontaneous puberty, only 50% percent of those complete puberty with menarche, and about a third of those will experience regular menstruation for many years. Spontaneous puberty is more often seen in girls with other karyotypes than 45,X and particularly those with mosaicism 45,X / 46 XX. Although the absence of genes on the X-chromosome in TS leads to gonadal failure, the precise mechanism is unknown. Besides the clinical features, girls with gonadal failure have an exaggerated biphasic pattern of gonadotropin secretion, with very high levels in infancy, declining to low values during mid-childhood and increasing by 9–11 yr to high LH and FSH levels. More recently, repeated low serum levels of Inhibin B and particularly low Anti Mullerian Hormone (AMH) levels seem to be better markers for gonadal failure in childhood in TS. In case of gonadal failure, 17 beta estradiol (oral or transdermal) has to be started at an appropriate age (11-12 years), with slow increase in dose over 3-4 years to induce puberty. Evidence suggests that starting with very low dose of estrogens at an even younger age will be even more favorable. Progestagens should be added after 2 years of estrogens to induced cyclic bleeding. To offer the best chance for fertility in adulthood, the recent further development of cryopreservation of egg cells may push the pediatric endocrinologist to collaborate even better with the gynaecologist already during the childhood of the girl with TS.

**WG5-78: Ovarian failure: the transition to adulthood**

*Gerard Conway*
University College London, Department of Endocrinology, London, United Kingdom

In 80% of women with Turner's syndrome, ovarian failure occurs before menarche and in this group careful consideration has to be given to the induction of puberty, sexual function and future options for fertility. In the remaining 20% of women with Turner's syndrome who retain apparently normal ovarian function, a strategy has to be in place for monitoring
ovarian reserve. Measurements of antimullerian hormone provide an effective method of stratifying future potential for fertility. The monitoring team must be up to date with new assisted reproduction technologies. For instance, it is now possible to offer young women with Turner's syndrome who have spontaneous menstruation, the option of oocyte cryopreservation. Whether to pursue this technology requires a great deal of discussion and as yet the success rate in terms of live healthy births is unknown.

WG5-80: Opportunity for fertility in Turner syndrome: oocyte cryopreservation and oocyte donation
Eleonora Porcu
Bologna, Italy

Turner syndrome is classified among the conditions of "premature ovarian failure" (POF). The number of germ cells is normal until the 18th week of gestation, after which begins the process of degeneration. During childhood, FSH and LH levels reach menopausal levels. Up to 30% of these patients show signs of pubertal development and 2-5% have regular menstrual cycles for a variable period of time. The spontaneous pregnancy occurs in approximately 2% of cases. In selected cases, fertility preservation can be proposed.

Fertility preservation can be achieved through different methods: egg donation, oocytes cryopreservation of mature or in vitro matured oocytes, embryo cryopreservation, cryopreservation of ovarian tissue.

The correct selection of patients suitable for fertility preservation represents a crucial point which is still under debate. Evaluation of ovarian reserve should be performed through non-invasive methods, such as basal serum FSH, AHM and inhibin B and ultrasonographic count of ovarian follicles. Normal hormone concentrations together with spontaneous pubertal development and mosaicism correlates with the likelihood of having ovarian follicles. In our experience, 1/3 of Turner patients were suitable for oocyte cryopreservation. When no eggs are available, the option of oocyte donation is associated with satisfactory pregnancy rates. However, a high rate of abortion and pregnancy complications such as premature delivery, IUGR, preeclampsia, maternal rupture or dissection of the aorta, are reported. A careful cardiological examination in these groups of eggs recipients is recommended. Moreover, it is particularly important to reduce the incidence of multiple pregnancies, by performing elective single embryo transfer.