האגודה הישראלית לאנדוקרינולוגיה IES Israel Endocrine Society

The 44rd Annual Meeting of the Israel Endocrine Society

April 14-15, 2015, Leonardo Hotel, Ashkelon

Program & Abstract Book

WELCOME ADDRESS

Dear colleagues;

It gives us great pleasure to welcome you to the 44th Annual Meeting of the Israel Endocrine Society IES. We started organizing this meeting right after the "Zuk Eitan" operation last summer, and as a token of our appreciation for the people's endurance, the venue of the 2015 conference is Ashkelon. This year we also welcome the integrated participation of our friends from the Israel Society for Calcified Tissues.

We would like to thank our distinguished lecturers from abroad, Clifford Rosen, Silvia Monticone, Shoshana Yakar and Kenneth McElreavey. We deeply appreciate their effort of coming despite our short notice invitation due to the circumstances and wish them a pleasant stay in sunny Israel.

As we always try to do, this year the program topics are meant to reflect the latest developments in clinical and basic advancements in endocrinology. To this end we shall enjoy the plenary presentations, attend a plethora of symposia lectures covering "from bench to bedside" topics, have meet-the professor discussions and enjoy the oral and guided posters presentations by our junior stuff and students. We will also have the traditional Prize session to encourage and emphasize excellent research.

We would like to thank all those involved in the organization of this meeting, the reviewers and the members of the Executive Committee for spending many hours making this conference interesting and enjoyable.

We wish you a successful conference.

Sincerely,

Yossi Orly

Chair - Organizing Committee

Yoav Sharoni

Co-Chair - Organizing Committee

IES EXECUTIVE COMMITTEE

Eddy Karnieli, M.D., President

Nehama Zuckerman-Levin, M.D., Secretary

Carlos Benbassat, M.D., Treasurer

Alon Chen, Ph.D.

Yona Greenman, M.D.

Avraham Karasik, M.D.

Rina Meidan, Ph.D.

Yoav Sharoni, Ph.D.

תודתנו נתונה לחברות נותנות החסות

על תמיכתן הנדיבה:

פרופ' הנס יוחנן לינדנר ז"ל – מילים לזכרו

פרופ' הנס יוחנן לינדנר נולד בשנת 1922 בגרמניה ועלה ארצה עם הוריו בשנת 1936. לאחר מלחמת השחרור הוא למד רפואה וטרינארית בסידני (אוסטרליה) וסיים בהצטיינות. את לימודיו לתואר Ph.D. הוא השלים באוניברסיטת קיימבריג' שבאנגליה. עם תום לימודיו, חזר לינדנר לאוסטרליה, התמנה כחוקר בכיר ב- (CSIRO) Commonwealth Scientific Research Organization והתרכז בחקר פיטואסטרוגנים. בשנת 1964, הגיע ארצה למכון ויצמן כחוקר אורח במח' לביודינמיקה.

כעבור שנה הוא קודם לדרגת פרופ' חבר ובשנת 1967 הוא מונה לראשות המחלקה.

פרופ' לינדנר בנה מחלקה מולטידיסיפלינארית שעסקה בחקר הפוריות ושינה את שמה ל: "חקר הורמונים".

בזכות תכונותיו התרומיות כאינטלקטואל וכמדען, נשא פרופ' לינדנר תפקידים רבים נוספים: הוא מונה במכון ויצמן כדיקן הפקולטה לביולוגיה, לראשות הועדה לקידום מדענים ולוועדה המייעצת של נשיא המכון. בנוסף לכך, הוא היה חבר בחבר הנאמנים של ביה"ח הדסה בירושלים, היה פעיל בהקמת הפקולטה לווטרינריה ואף היה נשיא האגודה הישראלית לאנדוקרינולוגיה. בתקופת כהונתו החלה מסורת קיום הכנסים השנתיים. פרופ' לינדר היה פעיל גם בארגונים בינל"א: חברת בועדות WHO, של מכון מקס פלאנק בגרמניה, של INSERM בצרפת, של ארגונים אנדוקריניים בינל"א וב-Editorial Board של עיתונים מדעיים. הוענקו לו תארי כבוד במס' אוניברסיטאות בעולם. בשנת 1979 הוענק לו פרס ישראל במדעי החיים והוא נבחר כחבר באקדמיה הישראלית למדעים. בשנת 1982 הוענקו לו פרס רוטשילד בביולוגיה וכמו כן, פרס Axel-Munthe בשטח הביולוגיה של הפוריות. פרופ' הנס יוחנן לינדנר נפטר בשנת 1982 עקב מחלה קשה. כראש המחלקה לחקר ההורמונים הכשיר פרופ' לינדנר דורות של חוקרים בתחום האנדוקרינולוגיה. הפרס ע"ש פרופ' לינדנר הוא הפרס היוקרתי ביותר של האגודה הישראלית לאנדוקרינולגיה. הפרס ניתן לחוקר/ת, מתחת לגיל 50 עבור הישגים מדעיים בתחום האנדוקרינולוגיה במהלך חמש השנים האחרונות.

זוכי פרס לינדנר

1989 – ישראל חנוקוגלו

1990 – מרדכי ליסקוביץ

1991 – ראובן רייך

1992 – אבי קרסיק

1993 – רוני זגר

1994 – עירית גרנות

1995 – אורי פלס

1996 – דורית אהרוני

1997 – חנה קנטי

1998 – בנימין גלזר

1999 – מיכל נאמן

2000 – רינה מידן

2001 – חיין ורנר

2002 – משה פיליפ

2003 – שרה פרבר

2004 – פואד פארס

2006 – איתן גרוס

2007 – אילן שמעון

2008 – חגית אדלר-פינקלמן

2009 – אסף רודיך

2010 – גיל ליבוביץ

2011 – אלון חן

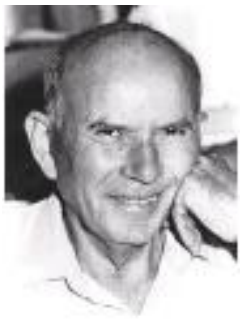
2012 – פיליפה מלמד

2013 – יובל דור

2014- ערן הורנשטיין

2015- איילת ארז

פרופ' ישראל חוברס ז"ל – מילים לזכרו



פרופ' חוברס נולד בפולין ב-1923 והגיע לארץ בגיל חצי שנה. את חינוכו היסודי קיבל בביה"ס החקלאי ע"ש מאיר שפיה. הוא היה פעיל במשך תקופה ארוכה בשורות ההגנה, בהבאת יהודים ארצה ובצה"ל. הוא התקבל ללימודי הרפואה בשוויץ, אך בינתיים פרצה מלחמת העצמאות והוא החליט להישאר בארץ ולהשתתף בה באופן פעיל, בעיקר בהגנת איזור ירושלים. עם גמר המלחמה, סיים את לימודי הרפואה באוניברסיטה העברית בירושלים.

פרופ' חוברס שרת כרופא בית במחלקת עצבים ולאחר מכן השלים את התמחותו כרופא פנימי במחלקה פנימית בהדסה. מתוך עבודתו ברפואה פנימית ובנוירולוגיה, החל פרופ' חוברס להתעניין באנדוקרינולוגיה ואף היה בין הראשונים שקיבל תואר רופא מומחה בשטח זה בארץ. הוא התעניין במיוחד בתחום הנוירואנדוקרינולוגיה שבו תרם רבות מבחינה עיונית ומחקרית.

בשנת 1962 יצא פרופ' חוברס מטעם NIH להשתלמות באוניברסיטת פנסילבניה, שם עבד בשיתוף עם פרופ' McCann שעבודתו הקנתה לו מעמד של חלוץ במחקר האנדוקריני בתחום הקשר בין ההיפותלמוס והורמוני יותרת המוח, ובעיקר בגילוי ובאפיון של הפקטור ההיפותלמי המזרז את הפרשת הגונדוטרופיניפ מיתרת המוח (מאוחר יותר, זיהוי סופי של פקטור זה כ-LHRH ע"י Shally הקנה לו פרס נובל).

עם שובו ארצה המשיך פרופ' חוברס את עבודתו במח' פנימית בביה"ח הדסה והועלה לדרגת פרופסור. במקביל לעבודתו כרופא, הוא הקים מעבדת מחקר לאנדוקרינלוגיה ניסויית במסגרת מחלקת עצבים. פרופ' חוברס וקבוצתו עסקו בחקר מנגנונים עצביים ואנדוקריניים הקשורים בויסות חום הגוף ובתפקיד מערכת העצבים המרכזית בויסות הפעלת הורמוני הדחק. כמו כן, עסקה מעבדתו בחקר יחסי הגומלין בין ההיפותלמוס האינסולין ורמת הגלוקוז בדם. מחקריו של פרופ' חוברס הקנו לו שם בינלאומי בתחום הנוירואנדוקרינולוגיה. הוא הוזמן להציג את מחקריו בפני כנסים בינלאומיים ושהה כמדען אורח באוניברסיטאות ובמכוני מחקר מהחשובים בעולם. לצד עיסוקו ברפואה, במחקר ובהוראה, מצא פרופ' חוברס זמן לתת שירותים רפואיים ללא תמורה לאוכלוסיה מעוטת יכולת בירושלים.

ב-1975 מונה פרופ' חוברס כמנהל המח' האנדוקרינית ומכון המחקר ע"ש רוגוף בביה"ח בילינסון. עם זאת, אהבתו לירושלים ולביתו בבית-זית ושאיפתו לעסוק ברפואה פנימית, על כל היבטיה, הביאו אותו לקבל את הצעת ביה"ח "ביקור חולים" לנהל את המח' הפנימית. על אף הקשיים הרבים שבהם היה נתון ביה"ח, ובמיוחד המח' הפנימית, הצליח פרופ' חוברס, בזמן קצר יחסית, לארגן צוות רופאים ועובדים ולשנות כליל את פני המחלקה. ביוזמתו עבר ביה"ח שינויים ניכרים לקראת הפיכתו לבית-חולים מודרני ואוניברסיטאי. במסגרת שיקום המחלקה, הקדיש פרופ' חוברס תשומת לב רבה לשטח האנדוקרינולוגיה ובמיוחד לנושא הסוכרת. הוא הקים יחידת סוכרת עם ציוד מודרני וייחודי להדרכה, אבחון, טיפול ומחקר קליני. במקביל לעבודתו בביה"ח ביקור חולים ,"מונה פרופ 'חוברס כמנהל השירות האנדוקריני של קופ"ח הכללית בירושלים. במסגרת זו הוא ארגן וניהל את מרפאת הסוכרת של קופ"ח בפרוז'ינין אשר סיפקה את שירותיה לאלפי חולי סוכרת במחוז י-ם.

פרופ' חוברס הקים וחינך דור של רופאים וחוקרים העוסקים ברפואה פנימית, אנדוקרינולוגיה וסוכרת. הוא הדגיש תמיד את חשיבות הגישה החמה לחולה ובמיוחד לחולה הבודד והקשה. פרופ' חוברס, שהיה מותיקי האגודה הישראלית לאנדוקרינולוגיה, נפטר באופן פתאומי ב-3.2.89, לאחר מותו, יסדה משפחתו פרס לזכרו לשם קידום המחקר האנדוקריני בישראל. הפרס מוענק לחוקר צעיר, מתחת לגיל 45 עבור עבודה בתחום האנדוקרינולוגיה שפורסמה בשנה האחרונה (או עומדת להתפרסם).

זוכי פרס חוברס

1992 – דניאל מלול

1993 – טלי נוה-מני

1994 – ליאורה שוקובסקי

1995 – איריס קרן-טל

1996 – קרן פז

1997 – פואד פארס

1998 – ארף רודיך

1999 – סיגל כורם

2000 – אפרת וורטהיימר

2001 – אלון חן

2002 – רינה המי

2003 – יעל קלמה

2004 - שלומי לזר

2006 – אמיר תירוש

2007 – נועה שר וערן גרשון

2008 – עירית מיבר-לוי

2009 – עידו וולף

2010 – מוריר חמאיסי

2011 – רעות אשואל

2012 – יעל קופרמן

2013 – יונית מרקוס

2014 – דנה חודרלנד

2015- יעל שרגא-לוי

Tuesday, April 14th 2015			
07:30-08:30	**Registration, Exhibition Visit, Gathering and Refreshments**		
08:30-10:15	Oral Presentations (3 Parallel Sessions)		
	Diabetes Mellitus	**Neuroendocrinology, Adrenal, HPA**	**Bone**
10:15-10:45	**Coffee Break, Exhibition Visit and Refreshments**		
10:45-11:00	Opening Remarks: Eddy Karnieli and Yossi Orly		
11:00- 11:45	**Plenary lecture 1: Clifford Rosen** **"What is New between Bone and Fat?"**		
11:45-13:15	Guided Poster Session		
13:15-14:15	**Lunch Break and Exhibition Visit**		
14:15-15:45	**Symposium 1-3 (Parallel Sessions)**		
Symposium 1: Bone Metabolism			
Clifford Rosen: "Metabolic Demands in the Bone Marrow- Why do we care?"			
Shoshana Yakar: "The Roles of the GH/IGF-1 Axis in Bone Metabolism during Development and Aging"			
Rivka Dresner-Pollak: "The Role of the Anti-aging Gene Sirtuin1 in Osteoporosis: From Mice to Women"			
Efrat Monsonego: "Nutritional Aspects of Skeletal Development"			
Symposium 2: Genetic Testing in Endocrine Tumors: Indications and Clinical Implications Regarding Prognosis and Treatment			
Ilan Shimon: "Pituitary Tumors"			
Sagit Zolotov: "Differentiated Thyroid Carcinoma"			
Etty Osher: "Pheochromocytoma and Paraganglioma"			
Symposium 3: Diabetes			
Gil Leibowitz: "Autophagy is a Major Regulator of Insulin Homeostasis in the Beta-Cell**"**			
Yehiel Zick: "Novel Regulators of ER Stress and the Unfolded Protein Response in Pancreatic Beta Cells"			
15:45-16:00	**Coffee Break and Exhibition Visit**		
16:00-17:30	**Symposium 4-5 (Parallel Sessions)**		
Symposium 4: Cutting Edge Molecular Technologies-Toward Personalized Medicine			
Noam Shomron: "Reading DNA (Whole Exome Sequencing) and RNA (Expression and Regulation) for Personalized Medicine "			
Tamar Geiger: "Application of Proteomics Technology to Cancer Research, from Diagnostics to Personalized Therapy"			
Symposium 5: Disorders of Sex Development (DSD)			
Kenneth McElreavey: Disorders of Sex Development			
Liat de Vries: "A Child with 46XY DSD – Should Sex Reassignment be Considered?"			
Ghadir Assad-Elias: "Persistent Mullerian Duct Syndrome: Clinical Dilemma"			
Anat Segev-Becker and Sarah Meisler: "Uterine Removal in Mixed Gonadal Dysgenesis / XY Partial Gonadal Dysgenesis, Raised as Males: Should we Leave it for Them to Decide?"			
17:30-18:30	**Meet the Professor / Expert (2 Parallel Sessions):**		
Naomi Weintrob: "Congenital Adrenal Hyperplasia: from Conception to Adulthood"	**Silvia Monticone**: "Clinical Approach to Primary Hyperaldosteronism (from Screening Tests to AVS)"		

Wednesday, April 15th 2015			
07:30-08:30	**Registration, Exhibition Visit, Gathering and Refreshments**		
08:30-10:15	Oral presentations (**3 Parallel Sessions**)		
	Growth Factors, Hormones and Cancer	**Reproduction**	**Thyroid**
10:15-10:45	**Coffee Break, Exhibition Visit and Refreshments**		
10:45-12:15	**Symposium 6 – 8 (3 Parallel Sessions)**		
Symposium 6: Parathyroid Disorders			
Iris Vered: "What is New in the New Guidelines for Primary Hyperparathyroidism?"			
Michal Mekel: "Imaging Detection of Parathyroid Adenomas"			
Auryan Szalat :"Disorders of the Calcium Sensing Receptor"			
Symposium 7: Hot Topics in Endocrine Research			
Noam Sobel: "Pheromones are Hormones too…"			
Oren Froy: "Effect of Meal Timing on Daily Metabolic Rhythms and Body Weight"			
Limor Landsman: "The Role of the Islet Micro-environment in Beta-cell Function"			
Symposium 8: Ovarian Failure and Function			
David Zangen: "XX DSD Gonadal Dysgenesis- New Genes and Mechanisms in Human Ovarian Development and Failure"			
Talia Eldar-Geva: " Can we Treat Primary Ovarian Failure ?"			
Nava Dekel: "Ovarian dendritic cells act as a double-edged pro-ovulatory and anti-inflammatory sword"			
12:15-13:00	**Plenary Lecture 2 : Silvia Monticone** **Recent Advances in the Pathogenesis of Primary Aldosteronism**		
13:00- 14:00	**Lunch Break, Exhibition Visit and Refreshments**		
14:00 -14:25	**Plenary Lecture 3: Erol Cerasi** **"Half a Century and a Bit in Diabetes"**		
14:25-16:00	**Prizes session and General Assembly** **Lindner Prize Lecture** (20 min) **Chowers Prize Lecture** (15 min) **Prizes for Best Clinical/Basic Abstracts** (10 min) **IES Members Assembly Meeting** (45 min)		
16:00-16:15	**Coffee Break**		

ORAL PRESENTATIONS

Bone

Boning Up on Bone Formation: Initial Stages of Calcium Phosphate Mineralization in the Larval Zebrafish Tail.

Anat Akiva[1], Karina Yaniv[2], Lia Addadi[1], Steve Weiner[1]
[1]*Structural Biology, Weizmann Institute of Science, Israel*
[2]*Biological Regulation, Weizmann Institute of Science, Israel*

In order to produce mineralized skeletons, many vertebrate groups first form a transient amorphous phase, which then transforms into the mature crystalline phase. An open question in biomineralization concerns the pathway through which ions are sequestered from the environment, transported, concentrated and aggregated within the soft tissues, to form the transient mineral phases. In bone, the nature of these transient mineral phases is also a subject of debate. Disordered calcium phosphate, amorphous calcium phosphate, polyphosphate and octacalcium phosphate (OCP), all are mineral phases that have been postulated to be involved in these transient phases during the bone formation process. Here we study calcium transport and mineral formation in the forming zebrafish larva caudal fin in vivo and ex vivo. We combine complementary imaging and spectroscopic techniques (confocal fluorescence and (cryo) scanning electron microscopy, correlative fluorescence-Raman microspectroscopy[1] and X-ray fluorescence) to show that the circulatory system is directly involved in calcium ion transport. We also show the presence of intracellular mineral-bearing vesicles located at the distal part of the tail prior to bone formation, and between forming fin bone hemi-cylinders. Some of the cells containing mineral-bearing vesicles also carry fluorescent markers for endothelial cells. Raman spectroscopy carried out on living fish, shows that the mineral deposited in the tissue between the fin ray bones is a combination of amorphous calcium phosphate and an OCP-like phase. The achievement of in vivo correlative confocal imaging and spectroscopy of cells and mineral, in a living animal advances our understanding of bone mineralization pathways in vertebrates.

1 M. Bennet, A. Akiva, D. Faivre, G. Malkinson, K. Yaniv, S. Abdelilah-Seyfried, P. Fratzl, and A. Masic, `Simultaneous Fluorescence-Raman Imaging of Bone Mineralization in Living Zebrafish Larvae`, *Biophysical Journal*, 106 (2014), L17-L19.

Seasonal Changes in Serum Ca, PTH and Vitamin D Levels in Patients with Primary Hyperparathyroidism

Anat Nevo Shor[1], Slava Kogan[2], Ben-Zion Joshua[3], Anat Bahat Dinur[3], Victor Novack[2], **Merav Fraenkel**[4]

[1]*Internal Medicine, Soroka University Medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel*

[2]*Clinical Research Center, Soroka University Medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel*

[3]*ENT, Soroka University Medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel*

[4]*Endocrinology, Soroka University Medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Israel*

Introduction: Primary hyperparathyroidism (PHPT) and vitamin D deficiency often co-exist in the same patient. There are seasonal changes in vitamin D levels which may also reflect on PTH and calcium levels. In this work we characterize seasonal changes in vitamin D, PTH and calcium levels in patients with PHPT. We further speculated whether these seasonal changes may have an effect on clinical decision making in patient with PHPT.

Methods: This was a retrospective study based on the electronic medical records of Clalit Health service in the south of Israel between 2000 and 2012. Patients 18 years and older with PHPT (PTHUpper limit of norm (ULN) and serum Calcium10.5 mg%) were included and all serum levels of calcium, PTH vitamin D were collected. Patients with renal failure or creatinine above 2 mg/dl were excluded. Seasons were defined as following: winter 1/12-30/3; spring 31/3-31/5; summer 1/6-30/9; fall 1/10-30/11.

Results: 844 patients, mean age 60±14.59 at diagnosis, met the laboratory criteria for PHPT in this time period and they had 15,151, 4448 and 2557 tests for serum Ca, PTH and Vitamin D respectively. Linear regression analysis showed that mean serum calcium and PTH levels were the highest in winter while vitamin D levels were the lowest in the winter. Logistic regression showed that the odds ratio (OR) for serum Ca11.5 mg/dl were highest in the fall (OR 1.3 P=0.003) while the OR for PTH 1.5 times the upper limit of norm and vitamin D

Conclusions: Serum levels of calcium PTH and vitamin D change with seasons in patients with PHPT. Since one of the criteria for parathyroidectomy is calcium more than 1 mg/dl above the upper limit which is more common in the fall, the season in which the patient is assessed may affect clinical decision making. It appears that in PHPT, PTH is more important than VitD in determining serum calcium levels.

Remodeling In Bone Without Osteocytes

Ron Shahar[1], Mason Dean[2], Ayelet Atkins[1], Lior Ofer[1], Laura Habegger[3], Philip Motta[3], Anna Shipov[1], Steve Weiner[4], John Currey[5]
[1]*School of Veterinary Medicine, Hebrew University, Israel*
[2]*Biomateials, Max Planck Institute, Germany*
[3]*Integrative Biology, University of South Florida, USA*
[4]*Structural Biology, Weizmann Institute, Israel*
[5]*Biology, University of York, UK*

A remarkable property of tetrapod bone is its ability to detect and remodel areas where damage has accumulated through prolonged use. This process, believed vital to the long-term health of bone, is currently considered to be initiated and orchestrated by osteocytes, cells within the bone matrix. It is therefore surprising that most extant fishes (neoteleosts) lack osteocytes, suggesting their bones are not constantly repaired, even though many species exhibit long lives and high activity levels, factors that should induce considerable fatigue damage with time. Here we show evidence for active and intense remodeling occurring in the anosteocytic, elongated rostral bones of billfishes (e.g. swordfish, marlins). Despite lacking osteocytes, this tissue exhibits a striking resemblance to the mature bone of large mammals, bearing structural features (overlapping secondary osteons) indicating intensive tissue repair, particularly in areas where high loads are expected. Billfish osteons are an order of magnitude smaller than mammalian osteons, however, implying that the nature of damage in this bone may be different. Whereas billfish bone material is as stiff as mammalian bone (unlike the bone of other fishes), it is able to withstand much greater strains (relative deformations) before failing, perhaps tied to its comparatively low mineral density and/or intricate architecture, both of which would tend to dissipate crack energy. Our data show that fish bone can exhibit far more complex structure and physiology than previously known, and is apparently capable of localized repair even without the osteocytes believed essential for this process. These findings challenge the unique and primary role of osteocytes in bone remodeling, a basic tenet of bone biology, raising the possibility of an alternative mechanism driving this process.

New Insights Into the 3D Hierarchical Organization of Human Lamellar Bone

Steve Weiner[1], Natalie Reznikov[1], Ron Shahar[2]
[1]*Structural Biology, Weizmann Institute, Israel*
[2]*Veterinary Medicine, Hebrew University, Israel*

Most of the human skeleton is formed of lamellar bone. The major components of lamellar bone are arrays of aligned mineralized collagen fibrils. Fibril orientation can vary within an individual lamella to form plywood-like structures. Using a dual-beam electron microscope and the Serial Surface View (SSV) method we elucidated the 3D organization of collagen [1]. The 3D study of human lamellar bone demonstrated the presence of ordered and disordered phases/materials, with the latter comprising 10-20% by volume of the bone. Furthermore, the disordered material in human lamellae forms a continuous 3D phase, which incorporates the entire lacuno-canalicular network. The disordered material is rich in non-collagenous organic material and may play, in addition to its structural role, a signaling and mechanosensing function in bone physiology. The ordered material of human lamellar bone is an anisotropic material characterized by dense packing. The detailed organization of the ordered sub-lamellar layers varies between individuals. At a higher organizational level, the ordered collagen fibrils are organized into 'rods' around 2 to 3 microns in diameter, and the long axes of these 'rods' are parallel to the lamellar boundaries [2]. The same structure is present in both compact and spongy bone. However, about half of the ordered component of the lamellar structure in a strut of spongy bone is co-oriented with the strut axis while the other half is co-oriented with the bone's anatomical axis. This new view of the 3D structure of lamellar bone necessitated a re-analysis of the overall hierarchical structural organization of bone [3].

[1] N. Reznikov, R. Shahar and S.Weiner. *Bone* **59**,2014, 93-104.

[2] N. Reznikov, R. Almany-Magal, R. Shahar and S.Weiner. *Bone* **52**, 2013, 676–683.

[3] N. Reznikov, R. Shahar and S.Weiner, Acta Biomaterialia **10**, 2014, 3815-3826.

Osteoclast Fusion is Initiated by a Small Subset of RANKL-Stimulated Monocyte Progenitors, which can Fuse to RANKL-Unstimulated Progenitors

Aner Ottolenghi[1], Mati Mann[2], Zvi Kam[2], Benjamin Geiger[2], Noam Levaot[1]
[1]*Physiology and Cell Biology, Ben-Gurion University of the Negev, Israel*
[2]*Molecular Cell Biology, Weizmann Institute of Science, Israel*

Bone resorption is achieved through the work of Osteoclasts. Osteoclasts are myeloidic cells closely related to macrophages. During differentiation some osteoclasts might undergo fusion to form a giant multi-nucleated cell (polykaryon). Although in recent years new proteins regulating osteoclast fusion have been discovered the cellular characteristics of osteoclast progenitors undergoing fusion is poorly defined. Using time lapse microscopy we show that in a given population of osteoclast progenitors some cells are more prone to fuse, assuming the role of fusion epicenters, which attract other, more passive, cells. Furthermore, while rate of fusion events involving polykaryons increases throughout the differentiation period, fusion events involving only mono-nucleated cells remains constant, suggesting a population of predisposed cells leading the fusion process (founders) and a population of cells being led (followers). In order to determine the distribution of founder cells we cultured osteoclast progenitors on micro-wells and scored for polykarions as cells with three or more nuclei. Our statistical analysis show that the fraction of founder cells in a given population of RAW264.7 differentiating osteoclasts is about 4% of the total progenitor population. Using a system we developed which allows us to track the origin of nuclei of two differently treated populations, we show that founder enriched RAW264.7 and primary osteoclast progenitor population (primed by 48 hours exposure to RANKL) can undergo fusion with cells which are not pretreated with RANKL, demonstrating that fusion is not directly linked to differentiation induced by the known cytokines.

These results and observations suggest the existence of a founder-follower mechanism. Understanding this mechanism may provide essential insights regarding the physiological role of fusion in normal, developmental and pathological states of bone.

Mechanobiology and mesenchymal stem cell differentiation fate

Dafna Benayahu
Cell and Developmental Biology, Sackler Faculty of Medicine, Tel Aviv University, Israel

Hormonal cues are important players in directing the lineage commitment of mesenchymal stem cells (MSCs). Recent studied add the attention for the importance of mechano-transductive mechanisms affects the stem cells activation and fate differentiation. The mechanotransduction is also part of the in the microenvironment cues relies on extracellular components' that affect the substrate rigidity. Such local signals lead to the interplay of MSCs in the musculoskeletal tissues and direct the cells' regulation into osteogenic / myogenic versus adipogenic lineage fate. The interest here is based on the understanding for inverse association between musculoskeletal cell function, the tissues strength and the adipose tissue appearance. It is recognized that an increase in number of adipocytes in skeletal tissue occurs with sedentary life style and in aging that also relies to tissue mechanical strength. The mechanical stimulation is translated from the physiology activity to the cells' via cell surface receptors and the cytoskeleton. The MSCs' sense and respond to the hormonal changes in aging or to the sedentary versus exercised environment. In our studies we measured the stem cells compartments i.e. the MSCs for osteogenic or satellite cells' from muscle tissues for their activation in vivo. Recent in vitro studies on adipogenesis mechobiology are focused on the role of both substrate stiffness and the mechanical stimulation. These studies bring perspective from in vivo and in vitro in understanding of the molecular regulation following mechanotransduction that underlines the MSCs lineage fate.

Systemically Injected Human Mesenchymal Stem Cells Home and Regenerate Osteoporotic Injured Vertebrae

Galina Shapiro[1], Dmitriy Sheyn[2], Wafa Tawackoli[2], Maxim Bez[1], Ilan Kallai[1], Eran Yalon[1], Elazar Zelzer[3], Tomer Stern[3], Zulma Gazit[1], Gadi Pelled[1], Hyun Bae[2], Dan Gazit[1]

[1]*Skeletal Biotech Laboratory, Hebrew University - Hadassah Medical Center, Israel*

[2]*Surgery, Cedars Sinai Medical Center, USA*

[3]*Molecular Genetics, Weizmann Institute of Science, Israel*

Osteoporosis-related vertebral compression fractures (VCFs) occur at a rate of 750,000 annually in the USA - twice the rate of hip fractures and have limited treatment options. It has been already established that recombinant parathyroid hormone (rPTH) can accelerate fracture repair in healthy animals by activating bone marrow mesenchymal stem cells (MSCs). However, osteoporotic patients might have decreased numbers and/or dysfunctional MSCs and hence rPTH would be less effective for fracture repair. Therefore, we hypothesized that an intravenous (IV) injection of MSCs combined with PTH therapy would induce stem cell recruitment to vertebral injury sites followed by enhanced osteogenesis and defect repair. In order to test the hypothesis, we created vertebral bone defects in osteoporotic Nude rats and treated them with IV injection of human MSCs and intermittent administration of rPTH. Our results showed that vertebral defects were rapidly and efficiently repaired when the animals were treated with a combination of MSCs and PTH, compared to either treatment alone or to no treatment at all (2.6 fold, p-value0.0001). Moreover, when we tracked labeled MSCs, we could determine that rPTH significantly enhanced cell homing to the lumbar region, where the MSCs differentiated to bone–forming cells. Immunohistochemistry results indicated that the CXCR4, SDF-1, EGFR and Ampheregulin play a role in the homing of MSCs to the vertebral bone defects. Finally, remarkable bone regeneration was observed when minipigs with multiple vertebral bone defects were treated with IV injections of allogeneic porcine MSCs and rPTH (2 fold, p-value

Food Restriction Increases Histone Deacetylase 10-Induced Autophagy in the Liver

Galit Pinto[1,2], **Galia Gat Yablonski**[1,2,3], Moshe Phillip[1,2,3]
[1]*Sackler School of Medicine, Tel Aviv University, Israel*
[2]*The Laboratory for Molecular Endocrinology and Diabetes, Felsenstein Medical Research Center, Israel*
[3]*Endocrinology and Diabetes, Schneider Children's Medical Center, Israel*

Background & Aims: Our previous data suggested that the histone deacetylase (HDAC) SIRT1 is involved in mediating the effect of nutrition on growth. The aim of the present research was to study the mechanism by which additional HDACs may be involved in nutrition-induced linear growth.

Methods: The *in vivo* studies were performed in young male Sprague Dawley rats that were either fed ad libitum (AL) or subjected to 10 days of 40% food restriction (RES) and then re-fed (CU). For in vitro studies, Huh7 hepatoma cells were used.

Results: Food restriction led to significant reduction in liver weight, concomitant with increased autophagy (i.e. a decrease in the levels of P62 and an increase in the expression level of AMBRA1 and ATG16L2 genes in the RES group). At the same time we found that the level of HDAC10 was significantly increased. Over expression of HDAC10 in Huh7 hepatoma cells led to reduced cell viability and increased autophagy as shown by increased conversion of LC3-I to LC3-II. An increase in the level of HDAC10 was also obtained when mTOR was inhibited by Rapamycin. siRNA directed against HDAC10 abolished the effect of Rapamycin on cell viability.

Conclusions: These results suggest that increased levels of HDAC10 may mediate the effect of malnutrition on growth attenuation and autophagy. Deciphering the role of epigenetic regulation in the nutrition–growth connection may pave the way for the development of new forms of treatment for children with growth disorders.

Complete TRMT10A Deletion in a New Syndrome of Failure to Thrive, Delayed Puberty, Intellectual Disability and Diabetes Mellitus

Amnon Zung[1], Michal Kori[2], Ella Burundukov[1], Tamar Ben-Yosef[3], Yasmin Tatoor[3], Esther Granot[4]

[1]*Pediatric Endocrinology Unit, Kaplan Medical Center, Affiliated with the Hebrew University of Jerusalem, Israel*

[2]*Pediatric Gastroenterology Unit, Kaplan Medical Center, Affiliated with the Hebrew University of Jerusalem, Israel*

[3]*Department of Genetics, The Rappaport Faculty of Medicine and Research Institute, Technion-Israel Institute of Technology, Israel*

[4]*Division of Pediatrics, Kaplan Medical Center, Affiliated with the Hebrew University of Jerusalem, Israel*

Objective: Two recent reports described a new syndrome of intellectual disability, short stature, microcephaly and young onset diabetes or disturbed glucose metabolism in association with inactivating mutations in the TRMT10A gene. TRMT10A gene encodes for tRNA methyltransferase that promotes post-transcriptional modifications of tRNA.

Case presentation: We investigated the clinical spectrum of a 17-year-old girl with TRMT10A deletion as part of contiguous gene deletion. From infancy she presented with failure to thrive and microcephaly. Puberty was characterized by slow and an inconsistent course of progression. Concomitantly, gonadotropin levels fluctuated between low and high levels which were compatible with gonadal failure. Unlike the previous reports, the patient had ketoacidosis at onset of diabetes and islet cell autoantibodies. Nevertheless, glycemic control was excellent (HbA1C 5.0%-6.2%).

Methods: TRMT10A expression was analyzed by RT-PCR and Western blot analysis. Endogenous insulin secretion and glycemic control were evaluated by glucagon stimulation test and continuous glucose monitoring. Glucose monitoring was performed both during insulin treatment and off therapy.

Results: Neither TRMT10A mRNA nor its translated protein were expressed in the patient, which confirmed the complete deletion of the TRMT10A gene. Endogenous insulin secretion still persisted 22 months after onset of diabetes, as shown by basal and glucagon-stimulated C-peptide levels of 671 and 1114 pmol/L, respectively. Relatively normal glucose levels were kept over three days without insulin treatment.

Conclusions: The patient`s diabetes shares features of both type 1 and type 2 diabetes. The fluctuating course of puberty and diabetes may reflect intermittent apoptotic damages due to sensitization of the relevant cells to various stress agents in the absence of functional TRMT10A.

Autophagy is a Major Regulator of β-Cell Insulin Homeostasis

Yael Riahi[1], Jakob D Wikstrom[1], Etty Bachar-Wikstrom[1], Nava Polin[1], Hava Zucker[1], Myung-Shik Lee[2], Peter Arvan[3], Erol Cerasi[1], Gil Leibowitz[1]

[1]*Department of Endocrinology, Hadassah Hebrew University Medical Center, Israel*

[2]*Department of Medicine, Samsung Medical Center, South Korea*

[3]*Division of Metabolism, Endocrinology, Diabetes, University of Michigan, USA*

Introduction: Pancreatic β-cells produce large amounts of insulin to maintain euglycemia. Autophagy is a lysosomal degradation pathway, which eliminates misfolded proteins to maintain cellular homeostasis.

Objectives: Studying the role of autophagy in regulating insulin production and secretion in the β-cell.

Methods: Insulin-like peptides (ILP) were localized in autophagic structures in pancreatic islets and INS-1E β-cells by electron and confocal microscopy. The effects of autophagy on proinsulin level and insulin secretion was analyzed by knockdown of the key autophagy genes, Atg5 and Atg7 or by pharmacological inhibition of lysosome acid hydrolase by bafilomycin-A1. ILP degradation was assessed by transfecting β-cells with wild type and mutant proinsulin tagged with GFP, followed by treatment with lysosome or proteasome inhibitors and Western blotting for GFP. Proinsulin content was assessed by ELISA and insulin secretion by static incubations at 3.3 and 16.7 mmol/l glucose.

Results: EM revealed dispersed ILP in autophagosomes in mouse pancreatic b-cells. Confocal microscopy showed that ILP are localized in vesicles, expressing the autophagosome/autolysosome markers LC3, P62/SQSTM1 and LAMP1. Knockdown of Atg5 and Atg7 increased proinsulin level. Similarly proinsulin content was increased, following treatment with bafilomycin-A1, whereas proteasome inhibitors had no effect. Further, proinsulin mutants that are irreparably misfolded and trapped in the ER were not degraded by lysosomes, suggesting that proinsulin is assigned for lysosomal degradation downstream to the ER. Colocalization studies suggest that this occurs at the trans-Golgi network. Intriguingly, we found that Chromogranin A, a large peptide secreted by most neuroendocrine cells is also robustly degraded by autophagy, suggesting that autophagy is a key regulatory pathway of hormone precursors in endocrine cells. Finally, inhibition of proinsulin degradation by inhibiting autophagy resulted in amplification of glucose- and KCl-stimulated insulin secretion.

Conclusion: Autophagy is a major regulator of insulin production and secretion and probably of other secretory peptides in the β-cell.

Potentiation of Insulin Response and Reduced Glycemia, Free Fatty Acids and Glucagon after Lunch and Dinner by Eating Vs Skipping Breakfast in Type 2 Diabetes

Daniela Jakubowicz[1], Julio Wainstein[1], Zohar Landau[1], Bo Ahren[3], Yosefa Bar-Dayan[1], Maayan Barnea[2], Oren Froy[2]

[1]*Diabetes Unit, Wolfson Med Center, Israel*

[2]*Institute of Biochemistry, Food Science and Nutrition, Hebrew University of Jerusalem, Israel*

[3]*Clinical Science, Lund University, Sweden*

Reduction of postprandial hyperglycemia (PPHG) is major target in treatment of type 2 diabetes (T2D). Skipping breakfast (B) has been associated with higher HbA1c and overall PPHG in T2D. We aimed to explore the effect of eating vs skipping B on PPHG after subsequent isocaloric lunch (L) and dinner (D). In crossover design 22 T2D, aged 57±1y; BMI 28.2±0.6 kg/m2; HbA1c 7.7±0.08 %, were randomly assigned to 2 meal test days: one day (YesB) with B, L and D, another day (NoB) the B was omitted. Postprandial plasma glucose, insulin, free fatty acids (FFA), glucagon and intact glucagon-like peptide-1 (iGLP-1) after isocaloric (700 kcals;% of CH:protein:fat:47:31:22) L and D were assessed.
AUC180 after Lunch in YesB vs NoB, was lower for glucose response (30566±19 vs 41804±31 mg/dl*min,p AUC180 after Dinner in YesB vs NoB, was less for glycemic response (37368±247 vs 47310±88 mg/dl*min,p AUC for glycemic response correlated positively with FFA (R2 = 0.78,p 0.0001) and with glucagon (R2=0.73,p 0.0001) and negatively with iGLP-1 (R2 = -0.79,p 0.0001) AUC responses after L and D.
Conclusion: Eating vs skipping B led to reduced PPHG after Lunch and Dinner. This was accompanied in YesB, by higher iGLP-1, higher and faster prandial insulin release and more suppressed plasma FFA and glucagon. Avoiding breakfast omission should be a strategy toward reduction of PPHG in T2D.

Regulation of β-Cell ER Stress by AMPK

Tal Israeli, Gil Leibowitz
Endocrinology and Metabolism Service, Department of Medicine, Hadassah-Hebrew University Medical Center, Israel

Background: Lipotoxicity constitutes a model for β-cell demise in obesity and type 2 diabetes. Exposure of β-cells to the fatty acid palmitate induces endoplasmic reticulum (ER) stress and apoptosis. During ER stress the transcription factor XBP1 is activated by splicing of its mRNA by IRE1α. The energy sensor AMPK regulates metabolic stress and inhibits mTORC1, an important regulator of the cellular response to stress. Our aim was to study the role of AMPK in the regulation of ER stress and apoptosis in β-cells under lipotoxic conditions and whether the AMPK effects on ER stress are mediated via mTORC1.

Methods: INS-1E β-cells were treated with and without palmitate and/or the AMPK activator AICAR. AMPK was inhibited using a dominant-negative AMPK adeno-virus (AMPK-DN). *Xbp1* splicing was inhibited using 4μ8C, a pharmacological inhibitor of IRE1α ribonuclease activity. Apoptosis was studied by Western blot for cleaved caspase 3 and by nucleosome ELISA assay. *Xbp1* splicing was measured by RT-qPCR. The involvement of XBP1 in the regulation of mTORC1 was tested in *Ire1α*- or *Xbp1*-knockout mouse embryonic fibroblasts (MEFs). Autophagy was assessed by Western blot for LC3-II and p62.

Results: Stimulation of AMPK attenuated palmitate-induced apoptosis and decreased *Xbp1* splicing in INS-1E cells, without affecting other markers of the unfolded protein response (UPR). Consistently, inhibition of AMPK using the AMPK-DN vector increased *Xbp1* splicing. Inhibition of *Xbp1* splicing by 4μ8C decreased palmitate-induced apoptosis. As expected, stimulation of AMPK inhibited mTORC1 activity. 4μ8C mimicked the effects of AMPK on mTORC1 both in INS-1E and MEF cells. This was accompanied by enhancement of autophagy. Surprisingly, 4μ8C inhibited mTORC1 also in *Ire1α* and *Xbp1* deficient MEFs, suggesting its effects on mTORC1 activity are not mediated via inhibition of *Xbp1* splicing.

Conclusions: Our study suggests that AMPK attenuates apoptosis in β-cells exposed to lipotoxicity; this protective effect is partially mediated via inhibition of *Xbp1* splicing by IRE1α and probably of mTORC1. The inhibition of mTORC1 by 4μ8C is independent of *Xbp1* splicing.

Sleep Quality and Risk of Diabetes and Coronary Artery Disease among Young Men

Gilad Twig[1,2,3], Avi Shina[1,3], Arnon Afek[6], Estela Derazne[1], Dorit Tzur[1], Tali Cukierman-Yaffe[2], Dalia Shechter-Amir[5], Hertzel Gerstein[4], Amir Tirosh[2,3]

[1]*The Surgeon General Headquaters, The Israel Defense Forces Medical Corps, Israel*

[2]*The Center for Endocrinology, Sheba Medical Center, Israel*

[3]*Talpiot Medical Leadership Program, Sheba Medical Center, Israel*

[4]*Division of Endocrinology & Metabolism, and Population Health Research Institute, McMaster University & Hamilton Health Sciences, Hamilton, ON, Israel*

[5]*The Sleep Institute, Sheba Medical Center, Israel*

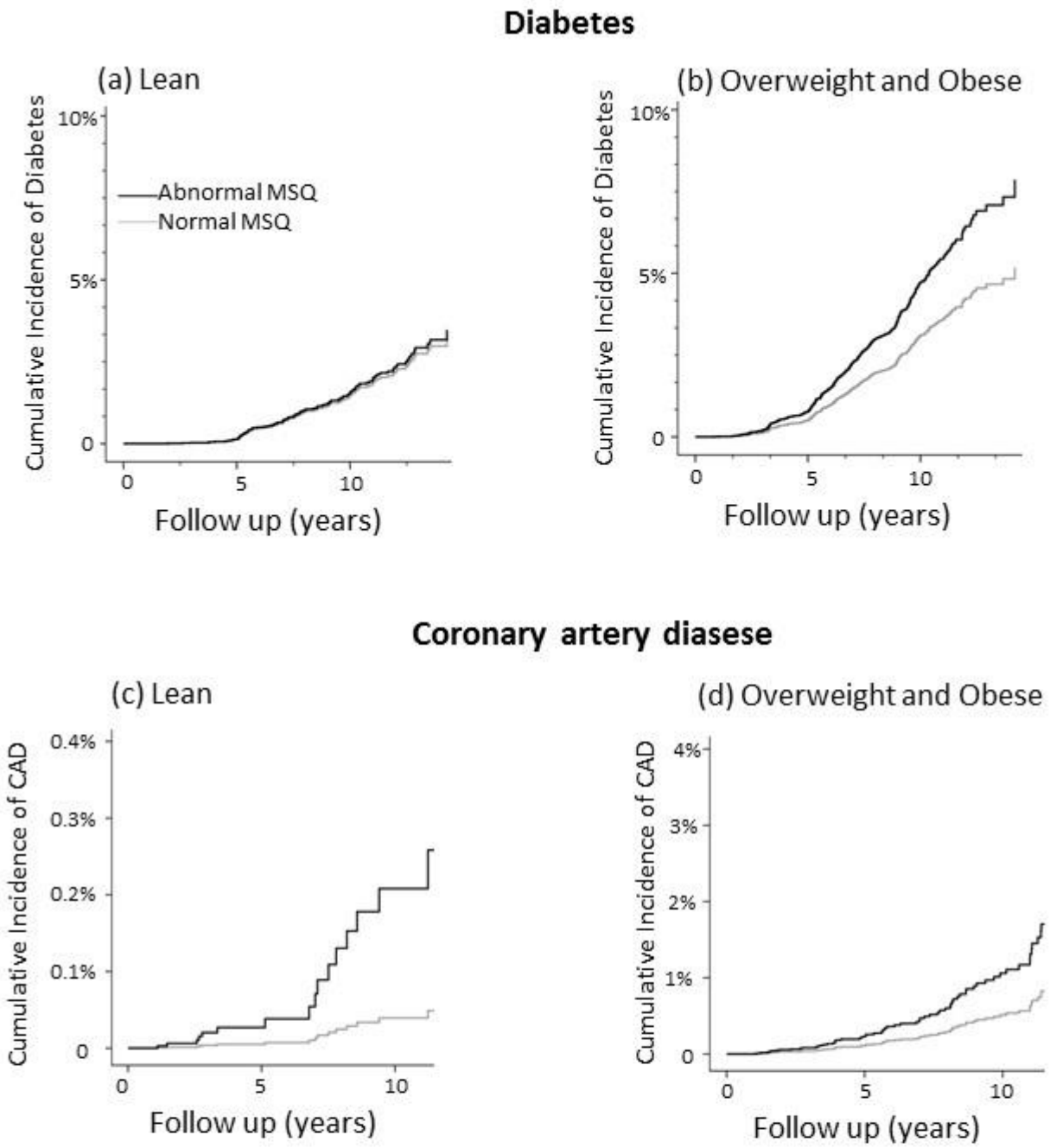
[6]*The General Manager Office, The Ministery of Health, Israel*

Aims/hypothesis: To assess the time-dependent effect of sleep quality on diabetes and coronary artery disease (CAD) incidence among young adults.

Methods: Incident rates of diabetes and CAD during a mean follow-up of 6.4±4.1 years were assessed among 26,023 men (mean age 30.9±5.6 years) of the Metabolic Lifestyle and Nutrition Assessment in Young Adults (MELANY) stratified by sleep quality at baseline, as assessed by the Mini Sleep Questionnaire (MSQ). Incident diabetes and CAD was analyzed using a Cox proportional hazard model.

Results: There were 445 cases of diabetes and 92 cases of CAD during 151,312 person-years. An abnormal MSQ score was associated with a 53% higher incidence of diabetes (95%CI=1.22-1.94, p0.001) compared to those with a normal score, after adjustment for age, BMI, fasting glucose, family history of diabetes, physical activity, smoking status, triglyceride level and WBC count. The increased risk associated with abnormal sleep quality remained when the MSQ score was modeled as a continuous time-dependent variable in a multivariable model (HR=1.04, 95%CI=1.02-1.05, p0.001). The increased risk was higher among overweight or obese participants (BMI and MSQ interaction p=0.046; figure a-b). Sustained abnormality in MSQ score resulted in higher HR for diabetes (2.35; 95%CI=1.56-3.52, p0.001). In addition, abnormal sleep quality was associated with a 2.38 higher incidence of CAD (95%CI=1.38-4.11, p=0.002), after adjustment for age, BMI, family history of CAD, smoking status, physical activity, blood pressure, HDL-c, LDL-c, triglyceride level and WBC count. As opposed to the effect on diabetes incidence, the effect of abnormal MSQ score was independent of obesity status (Figure c-d).

Conclusion/interpretation: Sleep quality contributes to the development of diabetes and CAD in apparently healthy young adults in a time-dependent manner. The use of a simple questionnaire to assess sleep quality may be a useful tool for risk stratification in this population.



25kg/m2), and overweight and obese BMI≥25kg/m2) young men in a multivariate model." width="720" height="960" /

Computer Model for Predicting Hypoglycemia Risk in Patients Hospitalized in Medical Wards

Yuval Barak Corren[1], Ram Ben-Mayor[2], Marianna Sherman[2], Avner Hatsek[3], Irit Hochberg[4]

[1]*Faculty of Medicine, Technion - Israel Institute of Technology, Israel*

[2]*Information, Computerization and Communications Department, Rambam Health Care Campus, Israel*

[3]*Medical Informatics Research Center, Department of Information Systems Engineering, Ben Gurion University, Israel*

[4]*Institute of Endocrinology, Diabetes and Metabolism, Rambam Health Care Campus, Israel*

Severe hypoglycemia during hospitalization in patients with diabetes is associated with increased risk of mortality. Concern about inpatient hypoglycemia is the main cause for undertreatment of hyperglycemia by hospital staff. Predicting which patients are at higher hypoglycemia risk could potentially reduce hypoglycemia events and improve patient safety.

We retrospectively analyzed a database of 14,731 hospitalization records of all adult diabetic or hyperglycemic patients (glucose over 200 mg/dL) hospitalized in medical wards in Rambam Health Care Campus during 2012-2013. Our case study included 766 hospitalizations with hypoglycemic events (glucose 70mg/dL) for which we matched 7,660 controls without hypoglycemia; controls were matched by baseline glucose level in the beginning of hospital admission. Data on demographics, diagnostic codes, Charlson score, drugs, and lab results (Glucose and Creatinine) were collected for each hospitalization.

Using Naïve Bayes classifier trained on 50% of the data (n=4,213) and tested on the remaining 50% we were able to predict 31% of the hypoglycemic events with a Positive Predictive Value of 26.2% and Negative Predictive Value of 93% (AUC 0.74). The factors that were most significant for this prediction were: lower glucose measurements and larger glucose variablity (SD) during the hospital stay prior to the event ;Intestinal infections (O.R 5.06 [2.5-10.2]); Septicemia (O.R 4.34 [2.2-8.6]); Creatinine greater than 2mg/dL (O.R 2.81 [2.38-3.31]); and Insulin or sulfonylurea treatment during the 24 hours prior to the hypoglycemia event (O.R 2.15 [1.83-2.51] and 1.76[1.38-2.24] respectively). Metformin and DPP4 inhibitor treatment were associated with reduced risk for hypoglycemia (O.R 0.62 [0.52-0.74] and 0.37 [0.19-0.73] respectively).

Utilizing this algorithm could help prevent inpatient hypoglycemia by increasing staff awareness to a higher risk in specific patients and indicate closer monitoring and use of lower medication doses.

Inadequate Nutrient Intake and Short Stature in Subject with Diagnosed Milk Protein Allergy

Tali Sinai[1], Roni Amitzur-Levy[1], Liat Nachshon[2], Michael Goldberg[2], Efrat Monsonego-Ornan[1], Yitzhak Katz[2]

[1]*Institute of Biochemistry and Nutrition, The Hebrew University of Jerusalem, Israel*

[2]*Allergy and Immunology Institute, "Assaf-Harofeh" Medical Center, Israel*

Rationale: A positive association has been reported between milk consumption and growth parameters. The majority of studies examining this association have been performed in children during their growth phase. We investigated the impact of a dairy-free diet on the final stature of IgE-mediated Cow Milk Allergy (IgE-CMA) young adults. These patients, by definition, are unable to consume even minor amounts of dairy foods, since infancy.

Methods: Anthropometric data was measured in 33 IgE-CMA patients (20.1±2.9 years old) and 25 healthy subjects (control group, 21.1±3.2 years old). Age- and gender-specific SDSs were determined according to Centers for Disease Control and Prevention growth charts. Nutrient intake assessment was based on 24 hour dietary recall and presented as percent of dietary reference values (DRI`s).
Individuals with conditions or treatments affecting bone metabolism or growth, were excluded.

Results: Growth parameters, including height, height-SDS, Weight-SDS and BMI-SDS were significantly reduced in CMA subjects when compared to controls (p0.05). An abnormal distribution of height-for-age was noted in the CMA group, as compared to the controls (48.5% versus 20.8% were categorized as less than the 25th percentile, respectively). In addition, height-SDS in CMA patients was significantly lower than their predicted height (mid-parental target height-SDS) (p0.001). The incidence of subjects consuming less than 67% of the DRI was greater in the CMA group, as compared to controls.

Conclusion: Individuals with CMA are at risk for not reaching their growth potential. Growth monitoring and appropriate dietary intervention may avoid nutritional deficiencies and growth retardation in these patients.

A Novel 75SIRT1-Dependent Survival Mechanism Activated Under Inflammatory Stress

Ashok Kumar, Yutti Daitch, Louisa Ben-Aderet, **Mona Dvir-Ginzberg**
Institute of Dental Science, Hebrew University of Jerusalem, Laboratory of Cartilage Biology, Israel

Previous reports in primary human chondrocytes show that pro-inflammatory cytokine stimulation leads to cathepsin B-mediated cleavage of the nuclear full-length SIRT1 (FLSIRT1; 110 kDa) to generate a stable but enzymatically inactive 75 kDa variant of SIRT1 (75SIRT1). 75SIRT1 was found to possess impaired chromatin binding in the nucleus and enhanced capacity to migrate to the cytoplasm, thus preventing apoptosis under proinflammatory stress. This research aims decipher the biochemical pathway and mechanism by which 75SIRT1 enhances cell survival under pro-inflammatory stress. Structural analysis of FLSIRT1 revealed a loop region (a.a. 520-551) which supported cathepsin B docking and cleavage of SIRT1. Using a deleted SIRT1 expression plasmid lacking the a.a. 528-543, cathepsin B mediated cleavage of SIRT1 was prevented. In chondrocytes, we previously found that 75SIRT1 binds cytochrome C and thereby enhances survival under stress by preventing cytochrome C assembly with the apoptosome complex. Here we predicted cytochrome C binds the N-terminus of 75SIRT1 (a.a.126-134, 150-166) using a prediction software. Immunoprecipitation assays using novel exogenous tagged 75SIRT1 and ΔNSIRT1 (lacking a.a. 1-240) expression plasmids were carried out to assess their capacity to bind cytochrome C. While 75SIRT1 possessed enhanced binding to cytochrome C, it displayed reduced binding to APAF1 resulting in enhanced survival under pro-inflammatory stress, which was not the case for cells expressing ΔNSIRT1. The results support that 75SIRT1 serves a functional role in cell survival by sequestering cytochrome C assembly with the apoptosome complex, under pro-inflammatory insult.

Cabergoline is an Effective Treatment or Clinically Non-Functioning Pituitary Adenomas

Yona Greenman[1,2], Iris Yaish[1], Odelia Cooper[6], Eyal Robenshtok[2,5], Nadav Sagiv[1], Tali Jonas-Kimchi[2,4], Jayati Mallick[7], Arkadiusz Gertych[7], Ilan Shimon[2,5], Zvi Ram[2,3], Shlomo Melmed[6], Naftali Stern[1,2]

[1]*Institute of Endocrinology and Metabolism, Tel Aviv Sourasky Medical Center, Israel*

[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

[3]*Department of Neurosurgery, Tel Aviv Sourasky Medical Center, Israel*

[4]*Neuroradiology Unit, Tel Aviv Sourasky Medical Center, Israel*

[5]*Institute of Endocrinology and Metabolism, Rabin Medical Center, Israel*

[6]*Pituitary Unit, Cedars Sinai Medical Center, USA*

[7]*Pathology Department, Cedars Sinai Medical Center, USA*

As optimal postoperative management of patients with clinically nonfunctioning pituitary adenomas (NFPA) is a matter of debate, the role of dopamine agonist (DA) therapy in this clinical setting was evaluated .

Methods: Retrospective analysis of prospectively collected data was conducted at two pituitary referral centers with different standard practices for post-operative management of NFPA: DA therapy or conservative follow up. Dopamine receptor 2 (D2R) expression was examined by immunohistochemistry, D2R long and short isoform mRNA expression was measured by quantitative RT-PCR.

Results: Seventy nine patients (mean follow-up, 8.8±6.5 years) were treated with DA, either initiated upon detection of residual tumor on postoperative MRI (preventive treatment- PT- group, N=55), or when tumor growth was detected during follow-up (remedial treatment -RT- group, N=24). The control group received no medication and comprised 60 patients (mean follow-up 6.3± 5 years).Tumor mass decreased,remained stable or enlarged respectively in 38.2%, 49.1% and 12.7% of patients in the PT group. Shrinkage or stabilization was achieved in 58.4% of the enlarging tumors in the RT group, whereas tumor growth persisted in only 41.6% of them. In contrast, tumor size enlarged in 53.3 % and remained stable in 46.7% of subjects in the control group (*P* 0·0001 for all comparisons).

15 year progression-free survival rate was 0·805 for the PT group, 0.48 for the RT group and 0.12 for controls; p0.0001(PT vs control), p=0.04 (RT vs control) and p=0.0053 (PT vs RT). 41.7 % of patients in the control group required additional surgery or radiotherapy as compared to 20.2% of the combined treatment groups (p=0.0084). There was no correlation between D2R expression and tumor response to DA treatment.

Conclusions: Dopamine agonist therapy is associated with decreased prevalence of residual tumor enlargement in patients with nonfunctioning pituitary adenomas, particularly when treatment is instituted prophylactically after surgical resection.

Founder Effect and the Clinical and Molecular Characteristics in a Cohort of Classical and Non-Classical Congenital Lipoid Adrenal Hyperplasia (CLAH) due to StAR Mutations

Abdulsalam Abu-Libdeh[1], Ariella Weinberg Shokrun[2], Ephrat Levy-Lahad[2], David Zangen[1]

[1]*Division of Pediatric Endocrinology, Hadassah Hebrew University Medical Center, Israel*

[2]*Medical Genetics Institute, Shaare Zedek Medical Center, Israel*

Introduction: Classical and non-classical congenital lipoid adrenal hyperplasia (CLAH) are caused by mutations in Steroidogenic Acute Regulatory Protein (StAR). The degree of enzyme activity impairment determines different clinical pictures.

Objective: To identify the genetic cause of Addisson's disease in a cohort of 11 unrelated families with classical and non-classical CLAH, correlate the clinical and molecular characteristics and to identify a possible founder effect of these mutations.

Results: All (n=14) affected individuals with classical CLAH in Israel and the Palestinian territories had the N-terminal c.201_202delCT mutation due to a founder effect and presented neonatally with severe Addissonian crisis and XY-DSD responding well to full replacement therapy. All (n=3) patients with non-classical CLAH had the G221S mutation (novel in the homozygous state), again with a founder effect. These patients presented during early childhood with addisonian crisis during a severe infection requiring just glucocorticoid replacement therapy. Characterization of the pubertal development in XX and XY patients of this cohort is underway.

Conclusion: Classical and non-classical CLAH due to StAR mutations are extremely rare but are significantly more common in the Palestinian population, given the founder effect of the 2 mutations characterized here. The different clinical phenotype of patients with classical and non-classical reflects the degree of StAR protein dysfunction caused by these mutations. To our knowledge, this is one of the largest cohorts studying the clinical and molecular characteristics of CLAH patients. The actual prevalence of mutations in the StAR gene in the general Palestinian population remains to be determined.

Pregnancy in Women with Non-Classical Congenital Adrenal Hyperplasia: Time to Conceive and Outcome

Ori Eyal[1,2], **Irit Ayalon**[2], Anat Segev-Becker[1,2], Anita Schachter-Davidov[1,2], Asaf Oren[1,2], Naomi Weintrob[1,2]

[1]*Pediatric Endocrinology, Tel Aviv Sourasky Medical Center, Israel*

[2]*Medicine Faculty, Tel Aviv University, Israel*

Background: Non-classical 21-hydroxylase deficiency (NC21OHD) is a mild form of congenital adrenal hyperplasia associated with different degrees of postnatal virilization. Elevated serum androgen concentrations have been reported to be a risk factor for infertility, early pregnancy loss and recurrent miscarriages in women with polycystic ovarian syndrome (PCOS).The aim of this study was to assess time to conceive and pregnancy outcome in NC21OHD women in correlation with glucocorticoid (GC) therapy and androgen levels.

Patients and Methods: We conducted a retrospective observational study in a tertiary medical center. The outcome of 130 pregnancies among 59 women (mean age at diagnosis 20 ± 9.5 years and mean age at first pregnancy 29 ± 5years) with biochemical and genetic diagnosis of NC21OHD was reviewed. Androgen and 17OHP levels were measured before and during each trimester. The mean GC dose was 7.4 ± 3.3 mg hydrocortisone/m².

Results: There was no difference in time to conceive between pregnancies of women with and without treatment (7.7 ± 11 months vs. 7.5 ± 25 months). There were 29 pregnancies without GC therapy and 101 with GC therapy. There was no significant difference between the rate of miscarriages between treated and untreated pregnancies (25% vs. 17%, respectively, P=0.6). Birth weight was significantly lower in GC-treated pregnancies compared to untreated pregnancies (2.9 ± 0.4 kg vs. 3.2 ± 0.5 kg, respectively, p=0.03). Androgen and 17OHP levels were similar in pregnancies that ended with miscarriage and those that ended with live birth.

Conclusions: Time to conceive is similar between treated and untreated pregnancies in NC21OHD women. In contrast to previous reports, there was no difference in miscarriage rates between treated and untreated pregnancies. There was significant decrease in birth weight in treated pregnancies despite the use of low doses of GC.

Hypothalamic CRFR1 Is Involved In Neurocircuits Mediating Energy Homeostasis

Meira Weiss[1,2], Yael Kuperman[3], Ofra Golani[4], Assaf Ramot[1,2], Alon Harmelin[3], Alon Chen[1,2]

[1]*Neurobiology, Weizmann Institute of Science, Israel*

[2]*Stress Neurobiology and Neurogenetics, Max-Planck Institute of Psychiatry, Germany*

[3]*Veterinary Resources, Weizmann Institute of Science, Israel*

[4]*Faculty of Biochemistry Biological Services Unit, Weizmann Institute of Science, Israel*

The arcuate nucleus (ARC) located in the mediobasal hypothalamus, plays a key role in regulating energy homeostasis and is composed of several neural populations, which mediate opposing effects on food intake and energy expenditure. The corticotropin-releasing factor (CRF) neuropeptide along with its specific receptor, CRFR1, play an important role in mediating the neuroendocrine and behavioral responses to stressful challenges. CRFR1 is highly expressed in the ARC, yet, the nature and function of these neurons are still unknown. In this study we identified and characterized CRFR1 expressing neurons in the ARC.

To this end we used a transgenic mouse model in which the CRFR1 neurons are genetically designed to express GFP. The different neural populations in the ARC were identified using either immunohistochemistry or by crossing the CRFR1-GFP mice with specific ARC-cell type reporter lines. Confocal microscopy of brain slices obtained from these mice demonstrates that the ARC-CRFR1 is expressed mostly (80%) by sub populations of orexigenic neurons, the agouti-related peptide (AgRP) and rat insulin promoter (RIP) expressing neurons, suggesting a crucial role for CRFR1 in regulating feeding behavior. In addition, injection of a viral vector encoding a floxed reporter to the PVN of CRF-Cre expressing mice, verified that ARC-CRFR1 neurons are innervated by CRF neurons arising from the PVN. We further characterized $AgRP^{+}CRFR1^{+}$ subpopulation, which constitute 45% of ARC-CRFR1 neurons. Using the AgRP-CRFR1 reporter mice we found that there are ~5000 AgRP neurons in the arcuate, 35% of which express CRFR1. Furthermore, we show that $AgRP^{+}CRFR1^{+}$ neurons are activated by stimulants of AgRP neurons, including fast and ghrelin and project predominantly to the LH, PVN and BNST brain nuclei.

These findings provide essential steps in elucidating the role of CRFR1 in mediating the metabolic consequences of stressful challenges.

This work is supported by the Perlman Family Foundation.

CRFR1 Activity Inhibits AgRP Neurons to Allow Appropriate Sympathetic Nervous System Activity Following Challenge

Elected as Best Basic Abstract

Yael Kuperman[1], Meira Weiss[2], Julien Dine[3], Katy Staikin[2], Assaf Ramot[2,3], Alon Harmelin[1], Jan Deussing[3], Matthiass Eder[3], Alon Chen[2,3]

[1]*Department of Veterinary Resources, Weizmann Institute of Science, Israel*

[2]*Department of Neurobiology, Weizmann Institute of Science, Israel*

[3]*Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Germany*

Stress and energy balance share common regulatory pathways orchestrated by hypothalamic neural networks. The corticotropin-releasing factor receptor type 1 (CRFR1), plays an important role in mediating the neuroendocrine and behavioral responses to stressful challenges. CRFR1 is highly expressed in the Arcuate nucleus (Arc) of the hypothalamus. We found that nearly half of CRFR1 neurons in the Arc colocalize with AgRP expressing neurons, yet the role of CRFR1 in these cells is unknown.

We used the Cre-lox system to knockout CRFR1 specifically in AgRP neurons. We found that this selective loss of CRFR1 expression leads to maladaptive thermogenic response to cold which is reflected in lower body temperature, reduced expression of uncoupling protein-1 in brown adipose tissue and reduced browning of the inguinal adipose tissue. In addition, mice lacking CRFR1 in AgRP neurons have reduced hepatic glucose production during fast due to reduced expression of key enzymes in the gluconeogenesis pathway. Our data suggests an inhibitory effect of CRF on AgRP neurons leading to reduced inhibitory effect on the second-order neurons. This is supported by electrophysiological recording which demonstrate that CRF application to $AgRP^{+}CRFR1^{+}$ neurons reduces their firing rate.

We propose a regulatory role for CRFR1 in a subset of AgRP neurons which enables appropriate activation of the sympathetic nervous system thus protecting the organism from hypothermia and hypoglycemia.

Plenary lecture 1:

What is New between Bone and Fat?

Clifford Rosen
Scarborough, Maine USA, Maine Medical Center Research Institute, USA

There remains significant debate about the relationship between obesity and osteoporosis. Obese individuals tend to have higher bone mass but in some cohorts, particularly older men, high BMI predisposes individuals to limb fractures; moreover many women in an English Registry who had osteoporotic fractures were obese. And T2D is associated with a greater fracture risk. However understanding the relationship between adipocytes and osteoblasts, both from a paracrine and endocrine perspective requires studies of type of fat depot and the skeletal compartment. In this talk, I will examine the relationship of white, brown and beige adipocytes to bone formation and resorption, and then delineate the interaction between marrow adipocytes and bone cells. Not surprisingly, all fat is not the same and its interactions with the skeleton are also both context specific and site dependent.

Metabolic Demands in the Bone Marrow: Why Should We Care? Clifford J Rosen MD

The bone marrow is a metabolically active compartment that is composed of multipotent progenitor cells, hematopoietic and vascular elements, as well as mesenchymal derived cells (MSCs), all of which require energy to differentiate and maintain homeostasis within the niche. Glucose is the preferred substrate for stem cells within the marrow but after entry into a differentiation scheme, metabolic fates change. It is well established that regeneration of hematopoiesis after transplantation and G-CSF demonstrates intense glucose uptake by PET-CT. In mice, after transplantation with prior irradiation there is death of recipient hematopoietic cells but ultimate resistance by mesenchymal elements. Intense NaF uptake by PET CT in the osteoid is followed by the appearance of marrow adiposity and subsequent hematopoietic repopulation. This model provides insight into substrate utilization by specific cells within the marrow, and in particular how osteoblasts use energy sources to make collagen. In this talk I will discuss lineage fate within the context of substrate utilization and how this might provide insights into novel therapeutics.

Group I - Adrenal, Thyroid, Neuro, Reproduction

Basal 17-Hydroxyprogesterone cannot Replace the ACTH Stimulation Test in the Diagnosis of non-classical Congenital Adrenal Hyperplasia in Children and Adolescents

Rachel Bello Vitrial[1], Yael Lebenthal[1,2], Ariel Tenenbaum[1,2], Moshe Phillip[1,2], Liat de Vries[1,2]

[1]*The Jesse Z and Sara Lea Shafer Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel, Israel*

[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

Objective: A basal 17-hydroxyprogesterone (17-OHP) serum level of 6.0 nmol/l has been suggested as a threshold for the diagnosis of non-classical congenital adrenal hyperplasia (NCCAH), particularly in children presenting with precocious pubarche (PP). We sought to determine if this threshold could lead to the underdiagnosis of NCCAH.

Design and Patients: A retrospective, cross-sectional study of 134 pediatric patients diagnosed with NCCAH (defined by a post-ACTH 17-OHP serum level 30 nmol/l).

Main Outcome Measure: The cohort was stratified according to basal 17-OHP (.

Results: Mean age at diagnosis was 8.2±4.1 years. Basal 17-OHP was p 0.03) and lower stimulated 17-OHP (p 0.001). Similarly, children referred for precocious pubarche with basal 17-OHP 6.0 nmol/l had lower serum levels of androstenedione (*p* 0.01), and stimulated 17-OHP (*p* 0.02).

Conclusions: A basal 17-OHP threshold of 6.0 nmol/l did not predict NCCAH accurately and carried the risk of overlooking its diagnosis. In children with a clinical presentation suggestive of NCCAH, the diagnosis should be based on an ACTH stimulation test.

Adrenal Medullary Hyperplasia: Case Report and Review of the Literature

Liat Barzilay- Yoseph[1], Orit Twito[1], Pnina Rotman-Pikielny[1], Ilan Leibovitch[2], Itshak Shechtman[3], Dan Nabriski[1]

[1]*Endocrinology, Metabolism and Diabetes Unit, Meir Medial Center, Israel*

[2]*Department of Urology, Meir Medial Center, Israel*

[3]*Department of Pathology, Meir Medial Center, Israel*

Introduction: Adrenal medullary hyperplasia (AMH) is a distinct entity, which may result in symptoms similar to those of pheochrocytoma. AMH, which can be hereditary or sporadic, is characterized by hyperplasia of the adrenal medulla in the absence of discrete tumor nodule.

Case report: A 54 year male was evaluated for metastatic disease including right kidney mass, left adrenal mass and femur metastasis. Laboratory evaluation revealed normal suppression of cortisol following 1 mg dexamethasone and normal levels of aldosterone and plasma renin activity. Urine collection revealed a significantly elevated normetanephrine/creatinin level of 1785 µg/gr creatinine (normal range 96-452). The patient recalled episodes of palpitations, sweating, weakness and elevated blood pressure during the past two years. He had tachycardia (100 bpm) and elevated blood pressure (150/100). The patient was prepared with phenoxybenzamine and propranolol. A right nephrectomy and left adrenalectomy were done. After surgery the patient blood pressure and pulse rate normalized without any medical treatment. Pathologic evaluation found metastasis of RCC to left adrenal, along with left AMH. The patient started sunitinib for his metastatic RCC.

Review the literature: Sporadic AMH is a clinicomorphologic entity that may mimic pheochromocytoma clinically and biochemically. Unlike pheochromocytoma there is no discrete tumor nodule in AMH. There is normal cellular architecture and decreased cortex to medulla ratio (less than 10:1). Hereditary AMH was identified in patients with germline *RET* mutation and in SDHB carriers. Laparoscopic adrenalectomy may be recommended as the gold standard treatment. Histology provides the definitive diagnosis.

Conclusions: We described a rare case of unilateral AMH in a patient with metastatic RCC. Removal of the affected adrenal cured the patient from hypertension and tachycardia. The association of RCC and pheochromocytoma is described in Von-Hippel Lindau disease, multiple endocrine neoplasia type 2 (MEN2) and SDHB mutations. Awareness to this rare pheochromocytoma-like disorder should be encouraged.

Pitfalls in Diagnosis of Primary Macronodular Adrenal Hyperplasia. A Case Report.

Irit Wirsansky Pearl, Leonard Saiegh, Mohammad Sheikh-Ahmad, Maria Reut, Jacob Bejar, Afif Nakhleh, Carmela Shechner
Endorinology, Bnei-Zion Hospital, Israel

Background: Assessing patients with Cushing`s Syndrome (CS) and low-normal plasma ACTH levels usually involves performing CRH stimulation test to differentiate between adrenal and pituitary etiology. Recently, there is growing evidence that primary macronodular hyperplastic adrenals can produce and secrete ACTH, with positive autocrine and paracrine effects on cortisol secretion.

Clinical case: A 56-yr-old man presented with cushingoid signs, developed over 5 years. Overnight dexamethasone suppression test (DST) – 4.05 mcg/ml, 24 hour urinary free cortisol (UFC) - 112 mcg/24 hr (N 0-115) and midnight free salivary cortisol– 0.24 mcg/dl (N 0-0.15). Basal ACTH level was in the low normal level– 3.08 pmol/l (N 1-10) and CRH stimulation test demonstrated 2-time elevation of plasma ACTH levels. Abdominal computerized tomography (CT) revealed a 2.8 cm right-adrenal nodule and a 1.4 cm left-adrenal nodule. Follow-up abdominal CT revealed enlargement of the right adrenal nodule up to 4.6 cm, with non-contrast density of 27 HU. Due to suspected adrenal malignancy, the patient underwent right adrenalectomy. Patho-histological examination revealed diffuse nodular and cortical hyperplasia, stained positive for ACTH. Post-surgical biochemical tests: DST- 1.23 mcg/dl, UFC – 88 mcg/24 hr, Elevated Basal ACTH level – 17.35 pmol/l, normal basal cortisol level – 13.6 mcg/dl, and normal ACTH stimulation test. Pre-surgical relatively good cortisol suppression on DST and positive CRH stimulation, did not support adrenal CS. However - histology, ACTH stains and post surgical remission were consistent with primary macronodular adrenal hyperplasia.

Conclusion: In CS, despite positive CRH stimulation, the coexistence of bilateral adrenal masses with low-normal ACTH levels, should raise the possibility of primary bilateral macronodular adrenal hyperplasia. As proposed by literature, adrenal venous sampling for ACTH levels may be considered in such cases.

Phosphoglucomutase -1 Deficiency Presented as Adrenal Insufficiency

Neta Loewenthal[1], Ruti Parvari[2], Alon Haim[1], Judy Tavashy[1], Eli Hershkovits[1]
[1]*Pediatric Endocrinology, Soroka University Medical Center, Israel*
[2]*Imunology and Genetics, Bengurion University Faculty of Health Sience, Israel*

Background: The congenital disorders of glycosylation (CDG) are a group of genetic diseases owed to defects in the biosynthesis of glycoproteins and other glycoconjugates. Phosphoglucomutase type 1(PGM1) deficiency is classified among the congenital disorders of glycosylation.
Phosphoglucomutase 1 catalyzes the interconversion of glucose-6-phosphate and glucose-1-phosphate ,Glucose-1-phosphate is an important intermediate in the pathways leading to protein Nglycosylation and in glucose homeostasis. Varied range of clinical manifestations recently described includes hepatopathy, bifid uvula, malignant hyperthermia, hypogonadotropic hypogonadism, growth retardation, hypoglycemia, myopathy, dilated cardiomyopathy, and cardiac arrest, ACTH deficiency has been reported but this finding is uncommon.

Objective and hypothesis: To report the clinical picture of 7 patients with PGM 1 deficiency from a consanguineous family presented with ketotic hypoglycemia .
Methods: Medical records of the patients were reviewed for clinical details and endocrine evaluation. Whole exome sequencing (WES) was performed.

Results: Seven patients ages between 2-29 are included, one patient died at 13 years old when gets off the school bus.
All patients have abnormal palatine structure (cleft palate, bifid uvula),4/7 had short stature) Pubertal development appeared normal including the older patient who fathered an affected patient. WES revealed previously described homozygous mutation c.112AT, p.Asn38Tyr in the PGM1 gene.

Conclusion: ACTH deficiency may be a common manifestation in patients with PGM1 deficiency having recurrent hypoglycemia.

Prolonged Adrenal Suppression after Adrenalectomy for Subclinical Hypercortisolism

Elena Chertok Shacham[1,2], Wasseem Rock[3], Helena Yodko[4], Avraham Ishay[1]
[1]*Endocrinology, Ha Emek Medical Center*
[2]*Internal Medicine E, Ha- Emek Medical Center*
[3]*Internal Medicine Department C, Ha Emek Medical Center*
[4]*Pathology Department, Ha Emek Medical Center*

Background: Subclinical Cushing syndrome is defined as a subtle cortisol overproduction in patients who don`t have the typical signs and symptoms of hypercortisolism. The growing interest in this entity is mainly due to extensive evaluation of incidentally discovered adrenal masses in order to exclude autonomous hormonal hypersecretion. The management of subclinical hypercortisolism is challenging because of lack of clear diagnostic parameters, the prevalence of obesity and other components of metabolic syndrome and the variability in glucocorticoid sensitivity due to polymorphisms of glucocorticoid receptors.

Methods: We describe a 45-year-old woman with a 6 years history of hypertension, partial thyroidectomy at age 9 due to adenoma, attention deficit disorder and depression. She underwent a comprehensive evaluation of hypertension including measurements of 24h urinary free cortisol on three occasions, two of them were slightly high and one was normal , plasma renin activity was low and aldosterone /renin ratio was elevated. An abdominal CT revealed a 3.5cm mass in her right adrenal gland consistent with cortical adenoma. Intravenous saline suppression test was normal with appropriate suppression of aldosterone. Urinary free catecholamines and metanephrines were performed twice to exclude pheochromocytoma . One mg dexamethasone suppression test revealed lack of appropriate suppression of cortisol. Midnight cortisol level was inappropriately increased. The diagnosis of subclinical Cushing`s syndrome was made. After discussing with her the options for further treatment, a laparoscopic adrenalectomy was performed. The histopathologic study confirmed a cortical adenoma. One week postoperatively she starts feeling unwell with increasing weakness, loss of stamina and more depressive mood. Adrenal insufficiency was diagnosed and she was started with replacement doses of cortisone acetate. Eight month after she was operated on, she still has very low cortisol levels after ACTH stimulation and treated with full replacement dose.

Conclusion: Severe and prolonged hypocortisolism may occur in patients with only subtle hypercortisolism. Therefore glucocorticoid coverage should be recommended to all patients after unilateral adrenalectomy in patients presenting with adrenal incidentaloma and subclinical hypercortisolism.

Cabergoline Treatment for Recurrent Cushing`s Disease During Pregnancy

Afif Nakhleh, Leonard Saiegh, Maria Reut, Mohammad Sheikh-Ahmad, Irit Wirsansky Pearl, Carmela Shechner
Department of Endocrinology, Bnai Zion Medical Center, Israel

Introduction: Cushing`s disease during pregnancy is associated with an increased risk for maternal and fetal complications. In recurrent Cushing`s disease following trans-sphenoidal surgery, and when re-operation is not feasible, medical treatment is usually considered. Cabergoline was found to be effective in reducing hypercortisolism in Cushing's disease. Evolving data concerning the safety of cabergoline use during pregnancy show no significant increase in the rate of complications during pregnancy or postnatal period.

Case report: We report a 29-year-old woman, Gravida 0 Para 0, with recurrent Cushing`s disease, three years after trans-sphenoidal resection of pituitary ACTH-secreting macroadenoma. The patient presented with a six-month history of weight gain, legs swelling, hirsutism, and irregular menses. Laboratory evaluation revealed: 24-h urinary free cortisol concentration of 131 mcg/24h (reference range: 20-90 mcg/24h), ACTH concentration of 22 pmol/L (reference range: 1.11-10 pmol/L), and serum cortisol, measured at 8:00 A.M. after 1 mg dexamethasone administration at 23:00 PM, of 6.2 mcg/dL. Repeated MRI revealed empty sella, with a small gadolinium-enhancing lesion, suspected to be an adenoma remnant on the medial wall of the right cavernous sinus. As the patient was not willing to undergo repeat surgical intervention, treatment with cabergoline was initiated, with a gradual dose titration up to 3.5 mg/week. Clinical improvement ensued, and four months later, she conceived spontaneously. After discussing treatment options with the patient, Cabergoline treatment at a dose of 2 mg/week was continued throughout pregnancy, during which the patient showed complete clinical remission. Consecutive tests of 24-h urinary free cortisol concentration were not found to be elevated. Pregnancy and delivery were uneventful except for mild hypothyroidism observed during the second trimester. At full-term the patient delivered a healthy female infant, by an elective cesarean section.

Conclusion: This case report demonstrates that cabergoline may be an effective and safe therapeutic option for the treatment of Cushing's disease during pregnancy.

Short and Long-Term Outcome of Differentiated Thyroid Cancer in Patients with no Lymph Nodes or Distant Metastases at Presentation.

Carlos Benbassat, Alex Gorshtein, Eyal Robenshtok, Ilan Shimon, Dania Hirsch

Endocrine Institute, Rabin Medical Center

Introduction: The presence of cervical lymph nodes (LN) and distant metastases are strong predictors of persistent disease in Differentiated Thyroid Cancer (DTC) patients. Yet, the majority of patients present with no LN disease (N0) and no distant metastases.

Aims: To investigate factors associated with outcome in DTC patients with no clinical evidence of LN metastases (cN0) and its comparison to patients presenting with LN disease (N1).

Methods: From the Rabin Medical Center Thyroid Cancer Registry we identified 246 patients with cN0 disease. This group was compared to 183 patients with pathologically proven disease (pN1) previously reported. All patients were operated after 1995. Patients with distant metastases at diagnosis were excluded, as were those with insufficient data for analysis. Total thyroidectomy and RAI was the initial treatment in 75% of cN0 and 100% of pN1 patients. Follow-up period was 8 years.

Results: Compared to patients with pN1 disease, patients with cN0 disease are more female and older, have smaller tumors, more unilateral and monofocal disease and less extrathyroidal extension (ETE). The persistency rate in cN0 patients was 9% compared to 44% in pN1 disease. Among cN0 patients there were no significant differences between those with (n=19) or without (n=194) persistent disease at one year for: age, gender, pathology type, ETE and I131 dose; but there was a difference for baseline stTg (48.7±47 vs 13.7±38 ng/ml, p=0.01) and size of primary tumor (21.9±17 vs 15.5±11, p=0.04).

Conclusions: Common risk factors distinguish between cN0 and pN1 disease, but are not enough to predict persistency among cN0 patients. Given the paucity of persistency in cN0 disease a much larger study is need to identify prognostic factors at this stage.

Impact of Gender on Outcomes in Differentiated Thyroid Cancer: A Matched Case-Control Study

Amit Akirov, Dania Hirsch, Carlos Benbassat, Ilan Shimon, **Eyal Robenshtok**
Endocrinology & Metabolism Institute, Rabin Medical Center, Beilinson Hospital, Israel

Background: Thyroid cancer is more common in women, but presents at a more advanced stage and carries a worse prognosis in men. Molecular factors that mediate this difference are generally attributed to estrogen levels and sex hormone receptors expression levels.

Objectives: To investigate whether gender disparity in DTC outcomes is due to differences in response to therapy between males and females who presented with the same initial disease staging.

Methods: A matched case control study of males versus females with DTC was conducted using Rabin Medical Center Thyroid Cancer Registry. Patients treated with total thyroidectomy and radioiodine (RAI) ablation were matched by age, histology, and TNM staging at presentation. Data on disease severity at presentation, treatment modalities, and outcomes were collected. Outcomes measures were evaluated at one year after initial treatment and at the end of follow-up.

Results: The cohort included 290 patients (145 male, 145 females). Baseline characteristics were similar, including age (49.6 vs. 50.1, NS), histology (papillary thyroid carcinoma 91% in both groups), and stage (47% stage I, 8% stage II, 22% stage III, 22% stage IV in both groups). Treatment was comparable with a mean radioiodine cumulative dose of 204±131mCi versus 183±134mCi (NS), and need for second surgery in 14/145 (9.7%) and 13/145 (9%) of males and females respectively. There was a small non-significant difference in disease status at one year with more persistent/recurrent disease in males (40.3% vs. 32.6%, p=0.2). This difference was larger at the end of follow-up, with worse disease free survival in males (68.5% vs. 77.5%, p=0.06) with borderline statistical significance.

Conclusion: Although male gender was associated with somewhat worse response to treatment, evidence is insufficient to support an independent role of gender in disease outcomes. Further studies are needed to establish this conclusion and evaluate gender adjusted approaches to therapy in DTC.

Preliminary experience with the AFIRMA Gene-Classifier Method in the Evaluation of Thyroid Nodules with Indeterminate Cytology

Iris Yaish, Karen Tordjman, Naftali Stern
Endocrinology, Tel Aviv-Sourasky Medical Center, Israel

Background: Indeterminate (Bethesda 3 and 4) cytopathologic results are reported in 15-30% of fine needle aspirations of thyroid nodules. Until recently, this inevitably led to surgery. However at surgery 70-80% of these cases turn out to be benign. ATA guidelines suggest the use of molecular markers for patients with indeterminate cytology, as a benign molecular pattern can justify observation rather than lobectomy. Among the available molecular approaches the AFIRMA©, gene classifier technique is reported to have a 94-96% negative predictive value.

Objective: To report on our initial experience with the AFIRMA -augmented FNA in nodules with indeterminate cytology.

Methods and Subjects: In recent months, we started to evaluate thyroid nodules with Bethesda score 3 or 4 by adding the AFIRMA technique on US-guided freshly obtained cytological samples Fifteen patients – 9 women and 6 men, mean age 48 y (range – 39-72) have so far undergone the procedure.

Results: Fifteen nodules, 9 Bethesda 3 (60%) and six Bethesda 4 (40%) were sampled. AFIRMA results are still pending for 3 samples. The AFIRMA technique yielded benign results in 6/12 nodules (50%), 5 were Bethesda 3, and only 1 Bethesda 4. Suspicious AFIRMA results were obtained in the 6 remaining nodules. Two out of the six (33%) were Bethesda score 3, and 4/6 (67%) Bethesda 4. Two patients with Bethesda 3 and suspicious AFIRMA results underwent surgery. Pathology was benign in 1 and consistent with papillary thyroid cancer in the other. Two patients with Bethesda 4 and suspicious AFIRMA recently underwent surgery, pathology results are still pending.

Conclusions: In our limited experience, The AFIRMA-augmented aspiration of thyroid nodules may indeed cut down on unnecessary surgery. This appears to be particularly the case with Bethesda 3 nodules. We are hoping to expand our experience with this technique, whose main obstacle remains its current cost.

Rate of Prolactin Suppression can Predict Future Prolactin Normalization, Tumor Shrinkage and Time to Remission in Male Macroprolactinomas.

Amit Tirosh[1,2], Carlos Benbassat[1,2], Ilan Shimon[1,2]
[1]*Endocrine Institute, Rabin Medical Center, Beilinson Campus, Israel*
[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

Introduction: Dopamine agonists are the mainstay treatment for prolactinomas, but clinical characteristics that predict their effects on prolactin (PRL) suppression and tumor shrinkage are missing. Our study aimed to find measures in early follow-up of men harboring macroprolactinomas, that will predict dynamics of PRL decrease and adenoma shrinkage.

Methods; A single center historical prospective study, including a consecutive group of 71 men with pituitary macroadenomas (10 mm) and hyperporlactinemia (7xULN), treated medically with cabergoline.

Comparisons of PRL normalization rates were performed according to PRL levels achieved at 6 months, maximal adenoma shrinkage during follow-up, and other patient characteristics. Correlations were performed to identify characteristics of PRL suppression dynamics.

Results: Five patients had nadir PRL levels x 3 ULN (51 ng/ml), and showed slower response to treatment with cabergoline with consistently higher PRL levels compared to other patients throughout follow-up (6 months mean PRL levels, 519±403 vs. 59±118 ng/ml, p0.001). PRL levels after 6 months of treatment correlated positively with current PRL levels (r=0.74, p0.001), with adenoma diameter following treatment (r=0.38, p=0.01), and with time to PRL normalization (r=0.75, p0.001). Shrinkage of adenoma size depicted by first MRI on treatment correlated with maximal adenoma shrinkage during follow-up (r=0.56, p=0.006).

Conclusions: Six months PRL level might serve as a surrogate marker for PRL normalization and adenoma shrinkage dynamics among men harboring macroprolactinomas. Among these patients with persistent hyperprolactinemia at 6 months, higher PRL levels are associated with resistance to cabergoline.

Pituicytoma- Case Report and Literature Review

Pinchas Klein[1], Mark Niven[1], Moshe Hadani[2]
[1]*Endocrinology and Diabetes Unit, Laniado Medical Center*
[2]*Department of Neurosurgery, Sheba Medical Center*

Case Report: A 52 year old male presented with hypogonadism. Laboratory assessment revealed panhypopituitarism with minimal hyperprolactinemia. MRI revealed a solid homogeneously enhancing 1.8 cm sellar mass extending into the suprasellar cistern with mild compression of the optic chiasm. Bitemporal hemianopsia was present. The lesion was suspected to be a non-secreting pituitary macroadenoma. At transsphenoidal surgery the lesion was noted to be very firm and could not be completely resected.

Histology revealed monomorphic spindle cells showing no mitoses. Ki-67 1%. Immunohistochemistry was negative for chromogranin, synaptophysin, EMA, GFAP, NF, pituitary hormones and positive for Vimentin, S100, TTF-1.

The surprising final diagnosis was pituicytoma.

Visual fields recovered completely after operation. However, he remained with panhypopituitarism requiring replacement therapy.

Imaging studies during the first year after surgery demonstrated a large although stable residual tumor, without significant compression of the optic chiasm.

Because of the proximity to the chiasm he underwent stereotactic radiosurgery.

Discussion: Pituicytoma is an exceedingly rare tumor with only tens of cases reported. It is a primary tumor of the neurohypophysis or infundibulum and may masquerade as a pituitary adenoma. It is considered a low grade glial neoplasm developing from pituicytes, modified glial cells.

Mean age of occurrence is around 50 with a slight male predominance.

Neuroimaging features are nonspecific and the diagnosis relies on histopathology.

Pituicytomas are typically indolent and treatment is surgical. Complete resection may be difficult due to high vascularity, firmness and adherence to surrounding structures.

Recurrence is common after incomplete resection. There are two main approaches after subtotal resection: immediate radiation therapy or active surveillance by MRI with radiation therapy reserved for aggressive tumor behavior.

Conclusions: Pituicytomas are rare tumors that mimic pituitary adenomas. It is important to recognize them because of differences in surgical approach, adjunctive therapy and follow-up.

Timing is Everything ? Postpartum Pituitary Dysfunction- Variability of Clinical and Radiological Presentation

Amit Tirosh[1,2], Hiba Masri Iraqi[1,2], Dania Hirsch[1,2], Eyal Robenshtok[1,2], Uri Yoel[3], Yoel Toledano[2,4], Orit Twito[2,5], Gloria Tsvetov[1,2], Ilan Shimon[1,2]

[1]*Endocrine Institute, Rabin Medical Center, Beilinson Campus, Israel*

[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

[3]*Endocrine Institute, Soroka Medical Center, and Ben Gurion University of the Negev, Beer Sheva, Israel*

[4]*Endocrinology Clinic, Division of maternal Fetal Medicine, Helen Schneider Hospital for Women, Rabin Medical Center, Israel*

[5]*Meir Medical Center, Endocrine Institute, Israel*

Introduction: Two etiologies are responsible for postpartum hypopituitarism; Sheehan`s syndrome which develops following traumatic labor, and lymphocytic hypophysitis. Since management of lymphocytic hypophysitis does not require surgery in most cases, pathology is usually absent, and the clinical presentation, in combination with laboratory and imaging characteristics establish the diagnosis. We aimed to describe the various patterns of presentation, including assisting analyses, among women with probable lymphocytic hypophysitis.

Methods: A retrospective study of women with pituitary dysfunction presented immediately or several months following delivery. Clinical characteristics data, pituitary hormone levels, and imaging findings were collected.

Results: Eight women were included; mean age at delivery was 34.8±7.4 years.

Most patients (6/8) presented with breastfeeding difficulty, and 5/8 reported headache. Among the patients with headache, 2/5 presented during pregnancy, and the others - 2, 4 and 9 months following delivery.

Hypopituitarism symptoms appeared immediately after delivery in half of the patients, between 7-12 months in 3/8; in one patient severe headache was the sole complaint.

All patients had central hypocortisolism, hypogonadotrophic hypogonadism, and growth hormone deficiency, and 7/8 had central hypothyroidism. Prolactin levels were low in 2/8. None of the patients had diabetes insipidus.

Five patients passed MRI within 3 months of symptom onset, four of them (80%) complained about headaches. These patients had either normal pituitary structure (2/5), pseudo-adenoma (1/5) or diffusely enlarged and hyperintense gland, suggestive for hypophysitis (2/5). Three patients have been diagnosed more than a year following presentation, all had reduced pituitary volume on MRI, and two of them had panhypopituitarism.

Conclusion: Over 300 cases of autoimmune hypophysitis were reported in the literature. Thus, a high index of suspicion is required to identify women in the postpartum period with breastfeeding difficulty, with or without headaches, and to study their pituitary function.

Vasorin Regulates Ovulation and Ovarian Reserve

Nitzan Rimon, Lia Yerushalmi, Dalia Galiani, Nava Dekel
Department of Biological Regulation, Weizmann Institute of Science, Israel

Bidirectional communication between the oocyte and the somatic cells is crucial for proper ovarian function. Many of the signaling molecules involved in this dialog belong to the transforming growth factor-β (TGFβ) superfamily. TGFβ signaling can be attenuated by its binding to the soluble form of vasorin/ATIA (Vasn), a type I membrane protein. In addition, Vasn is expressed in the mitochondria, where it protects cells from TNFα and hypoxia-induced apoptosis. Our main goal is to explore the role of Vasn in ovarian physiology and female fertility. We initially demonstrated that Vasn mRNA is expressed in mice granulosa cells (GC) at different stages of folliculogenesis. We also showed that Vasn expression is up-regulated in ovarian granulosa cells of pre-ovulatory follicles in response to luteinizing hormone (LH), further suggesting a mediatory role for EGFR and ERK1/2 signaling in this LH-induced Vasn expression. Examination of a Vasn systemic knock out mouse model revealed that the size of the primordial follicles population, which constitute the ovarian reserve, was more than double in these mice as compared to wild type. We generated Vasn conditional knock out (cKO) mice, in which Vasn was selectively deleted in the GC. Upon stimulation of these cKO mice by gonadotropins we found that the number of ovulated oocytes was significantly higher than that in their wild type (WT) siblings. In agreement, their GC expressed higher levels of genes involved in steroidogenesis and mitochondrial function. Higher progesterone levels were observed in naturally cycling cKO mice. However, the litter size in these cKO was not different than that in their WT siblings. These results suggest that Vasn plays a central role in regulation of ovulation and possibly also in the establishment or maintenance of the ovarian reserve. The specific involvement of this molecule in ovarian physiology is subjected to additional investigation.

PKA and PKC Differentially Regulate PGE2 and Progesterone Levels in Rat Immortalized Granulosa Cells Cultures

Alaa Nemer, David Ben-Menahem
Clinical Biochemistry and Pharmacology, Ben-Gurion University of the Negev, Israel

Production of inflammation-related molecules is typical to the preovulatory follicle, and isolated follicular cells from various species secrete prostaglandins in response to gonadotropins. Here, we examined PGE_2 secretion from immortalized granulosa cells expressing gonadotropin receptors. The cells were established by Abraham Amsterdam and his colleagues who isolated granulosa cells from preovulatory follicles of PMSG primed immature rats, immortalized the primary cells, and transfected them with rat LH or FSH receptor cDNA. These cells (denoted as LHR and FSHR cells) produce progesterone in response to gonadotropins. Here we examined the synthesis of PGE_2 in these cells. The cells were stimulated with gonadotropins, forskolin (an activator of the PKA pathway), dBcAMP (a cell permeable cAMP analog) and TPA (a PKC activator) alone and in combination for 24hrs, and PGE_2 and progesterone accumulation in the culture media was determined. We also used PKA and PKC inhibitors (H89 and GF-109203X). In agreement with previous studies, while gonadotropins and forskolin induced progesterone accumulation in the media, TPA did not. In contrast, our data indicate that TPA, but neither goandotropins nor forskolin or dBcAMP evoked PGE_2 accumulation in the media. Western Blot analysis suggested that this was in correlation with COX-2 levels in the cell lysate. While H89 reduced the gonadotropins and forskolin effect on progesterone secretion, GF-109203X attenuated the TPA-induced PGE_2 accumulation in the culture media of LHR and FSHR cells by 40-70%. Furthermore, gonadotropins and forskolin inhibited the TPA-induced PGE_2 production in both cell types (up to 65% reduction). These data suggest that PKA and PKC have opposite effects on PGE_2 and progesterone synthesis in these cells. We propose that the immortalized LHR and FSHR cells have features of differentiated granulosa cells, in which such PKA/PKC interplay on PGE_2 synthesis is characteristic to luteinized follicular cells.

Genomic Profiling of Bovine Corpus Luteum Development

Sigal Kfir[1], Noa Wigoda[1], Irit Orr[2], Shifra Ben Dor[2], Rina Meidan[1]
[1]*Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel*
[2]*Bioinformatics and Biological Computing Unit, Weizmann Institute of Science, Israel*

The corpus luteum (CL) is a transient endocrine gland involved in establishment and maintenance of pregnancy due to production of P4. The new CL develops from cells that remain in the follicle following ovulation but is eventually composed of multiple cell types including steroidogenic cells and non-steroidogenic cells (endothelial cells, pericytes, fibrocytes, and immune cells). To gain insight into the global changes that occur in the CL during its maturation the transcriptomes of early (day-4) and mature (day-11) CL were analyzed and compared by microarray. 681 genes were differentially expressed between 4 vs. 11 days, with 363 upregulated in 4 days, and 318 upregulated at 11 days. Using the DAVID and Ontologizer 2.0 annotation tools Gene Ontology (GO) analysis was performed (Table). These GO terms indicate that cell division is the main process in the early CL while immune activity is prominent in the mature CL and ready to be recruited during luteal regression. Next, we searched for specific cell markers and noted their expression in early and mature CL Most endothelial cell and pericytes markers, for example SelectinE, NOS3, desmin and PDGF-receptor1 were expressed in the day-4 CL demonstrating that the angiogenic process is complete 3 days after ovulation. Genes encoding steroidogenic enzymes did not differ between days. However, cholesterol synthesis, steroidogenic cell activators and their signaling were higher on day 11, thus defining the plausible reason for maximal progesterone production on day-11. This study sheds light on the immense changes that occur in the CL within a week's time and may lead to identification of new genes that play a role in CL maturation and function.

DAY-4

Count	%	p-value	GO Term
40	13.37793	2.52E-23	cell cycle
30	10.03344	2.34E-14	chromosome
27	9.0301	1.84E-11	DNA metabolic process
43	14.38127	3.47E-06	nucleoside binding
17	5.685619	1.40E-11	DNA replication

DAY-11

Count	%	p-value	GO Term
49	18.35206	2.51E-12	extracellular region
9	3.370787	2.24E-04	lysosome
15	5.617978	1.09E-03	immune response
24	8.988764	3.24E-06	lipid metabolic process
6	1.8867	0.009172	complement activation

Clinical Criteria Remain Paramount for the Diagnosis of Polycystic Ovary Syndrome in the Adolescent Age Group

Adi Weinberg[1], Moshe Phillip[1,2], Liat de Vries[1,2]
[1]*The Jesse Z and Sara Lea Shafer Institute for Endocrinology and Diabetes, Schneider Children's Medical Center of Israel, Israel*
[2]*Sackler Faculty of Medicine, Tel-Aviv University, Israel*

Context: Adolescent polycystic ovary syndrome (PCOS) may be difficult to distinguish from pubertal changes and diagnosis remains a challenge.

Objectives: To investigate the value of different biochemical parameters for the diagnosis of PCOS and also to assess the prevalence of non-classical congenital adrenal hyperplasia (NCCAH) among adolescent girls referred for clinical symptoms suggesting PCOS.

Design and Setting: A retrospective, cross-sectional study at a tertiary pediatric endocrinology unit.

Methods: Data of 114 girls aged 13-18 with a clinical presentation suggesting PCOS were analyzed, including results of basal androgens, prolactin, GnRH and ACTH stimulation tests, and pelvic ultrasound. Clinical and laboratory characteristics of girls diagnosed with PCOS (based on Rotterdam criteria) were compared to those of girls having "Isolated" hyperandrogenism or menstrual irregularities ("non-PCOS").

Results: Of the 114 girls, 9 (7.9%) were diagnosed with NCCAH and 87 with PCOS; 18 were non-PCOS. Compared to non-PCOS girls, those with PCOS had a significantly higher prevalence of hirsutism (p 0.002), PCO morphology by ultrasonography (P 0.001), menstrual irregularities (p0.001) and acne (p0.001). Precocious puberty was documented in 15 (17.4%) girls with PCOS and in none of the non–PCOS girls. Androstenedione, (p

Conclusions: Increased basal LH and basal LH/FSH ratio may support the diagnosis of PCOS in adolescents; however, the GnRH stimulation test is not contributory. Given the significant prevalence of NCCAH among adolescents presenting as PCOS, an ACTH test should be included in the work-up, at least in populations with higher prevalence. Since no one parameter is diagnostic for PCOS, clinical criteria remain paramount.

Performance of Dexamethasone-Suppressed Corticotropin-Releasing Hormone Stimulation Test in Morbid Obese Adults

Afif Nakhleh[1], *Leonard Saiegh*[1], *Maria Reut*[1], *Mohammad Sheikh-Ahmad*[1], *Limor Chen-Konak*[1], *Irit Wirsansky Pearl*[1], *Carmela Shechner*[1]

[1] *Department of Endocrinology, Bnai Zion Medical Center, Haifa, Israel*

Background: In order to differentiate between Cushing`s syndrome (CS) and Pseudo-cushing`s syndrome, it is customary to use the test that is conducted by suppression of cortisol with low-dose dexamethasone followed by the administration of corticotropin releasing hormone (Dex-CRH test). In children with severe obesity, Dex-CRH test has shown a specificity rate of just 55%.

Objective: The aim of the study was to evaluate the specificity of Dex-CRH test in morbid obese adults.

Study design: The study included a total of 19 subjects with body mass index (BMI) equal or higher than 40 kg/m2, who were referred to obesity outpatient clinic in the course of evaluation for bariatric surgery. In all subjects Dex-CRH test was performed, and 24h Urinary free cortisol was collected prior and during the second day of dexamethasone administration (2nd-day-UFC).

Results: BMI was 45.1 ± 4.6 kg/m2 in women and 45.7 ± 3.3 kg/m2 in men respectively. 14 subjects underwent bariatric surgery. No subject had surgical or perioperative complications and all surgically treated subjects had improvement of metabolic status and mean body weight loss of 46.5 ± 16.6 kg. All except for 2 subjects had normal Dex-CRH test, with 15-min cortisol falling below 1.4 ug/dl. During follow-up, no subject gained additional weight, neither developed signs of CS. 15-min-cortisol concentration of 1.4 ug/dl revealed a specificity of 89% and 2nd-day-UFC of 16 ug/24h showed a specificity of 100%.

Conclusion: Morbid obesity in adults seems not to comprise a significant confounder in Dex-CRH test, and 15-min-cortisol concentration of 1.4 ug/dl had a higher specificity than previously reported in obese children.

Mechanical Stimulation Effects on Adipogenesis

Lisa Mor-Yossef Moldovan, Dafna Benayahu, Efrat Kaisler, Amit Gefen
Cell and Developmental Biology, Sackler Faculty of Medicine, Tel Aviv University, Israel

Adipocytes differentiated from mesenchymal stem cells under the regulation of hormonal factors. Adipocytes play a critical role in several pathophysiology including obesity, osteoporosis and osteopenia. The increased number of adipocytes in skeletal tissue is recognized in aging and along sedentary life style that relates to tissue mechanical strength. Here, we present a new view that follows the mechanical stimulation on adipogenesis.

The *objectives* of the study were to assess in *in vitro* system i) The fate for cells' cultured on rigid and flexible substrates and ii) The differentiation capability for cultured adipocytes on flexible substrate under applied static mechanical stimulation.

Methods*:* Preadipocyte cells were grown on flexible membranes to allow mechanical stretching. The adipogenesis was recorded by digital photographs and analyzed by MATLAB for cells' morphology and lipid droplets (LD) accumulation. We also followed the proteins expressed during adipogenesis by immunofluorescence (IF) and aimed to use Mass spectrometry for potential discover new proteins that are regulates this pathway.

Results*:* We demonstrated that cell stretching and substrate rigidity affected pre-adipocyte differentiation. The substrate rigidity affected the forces involved in cell adhesion, leading to changes in cell morphology. The mechanical stimulation applied to flexible substrate increased the adipogenesis as compared to non-stretched cultures. Using MATLAB we measured the radius and number of LDs per cell. The results demonstrated increased LD radius under static mechanical stimulation. Also cells grew under non-differentiation medium expressed "spontaneous" adipogenesis presumably via cell-cell and stretching. To follow the cell fate changes we used the IF for known proteins, such as cytoskeleton (actin and tubulin), the modulation of transcription factors [PPARγ, glucocorticoid receptor (GR), chromatin remodeling protein 9 (CHD9)]. Hence, the current study brings new perspective in understanding of the molecular regulation of mechano-transduction that underlines adipogenesis.

Increased Bone Mass and Bone Anabolic Functions in Mature GPR39 Deficient Mice

Milena Pesic, Gali Guterman Ram, Ayelet Orenbuch, Noam Levaot
Physiology and Cell Biology, Ben-Gurion University of the Negev, Israel

Bone is a dynamic structure which undergoes constant bone remodeling. Bone remodeling is regulated by a balance between osteoblastic formation and osteoclastic resorption. Zinc, an essential trace element, is important regulator of bone homeostasis. Recently, a receptor specifically activated by zinc was identified. This receptor named GPR39 belongs to the G Protein Coupled Receptor (GPCR) superfamily. However, a role of GPR39 in regulation of bone metabolism was not explored. In order to elucidate a role for GPR39 in bone metabolism we investigated the bone phenotype of GPR39 deficient mice. These mice had normal body length and weight. However, micro CT analysis of trabecular bone in the femurs of six month old mice revealed a significant 32% increase in trabecular bone fraction compared to wild type littermates. Increased trabecular bone in these mice was a result of higher trabecular number and trabecular thickness. In order to test whether increased bone mass was a result of attenuated resorption by osteoclasts we analyzed serum levels of CTX-1 peptide, a marker for bone resorption. CTX-1 levels in GPR39 deficient mice were lower but did not reach statistical significance. On the other hand, serum levels of PINP-1, a marker for bone formation, showed a significant 26% increase in GPR39 deficient mice indicating an increase of bone formation. We compared the mineral secretion of osteoblasts isolated from bone marrow of GPR39 deficient and wild-type mice. The intensity of mineral staining was higher in cultures of GPR39 deficient osteoblasts suggesting an increased function of GPR39 deficient osteoblasts.

Our data reveal a novel role for the zinc receptor GPR39 in regulation of bone homeostasis and suggest that its absence leads to excessive bone formation by osteoblasts.

Pregnancy Outcomes in Women with Primary Hyperparathyroidism

Dania Hirsch[1,5,6], Vered Kopel[2], Varda Nadler[2], Sigal Levy[3], Yoel Toledano[4,6], Gloria Tsvetov[1,5,6]

[1]*Institute of Endocrinology, Rabin Medical Center*
[2]*Central Laboratory, Maccabi Health Care Services*
[3]*Sackler Faculty of Exact Sciences, Tel Aviv University*
[4]*Maternal Fetal Medicine Division, Rabin Medical Center*
[5]*Sackler Faculty of Medicine, Tel Aviv University, Israel*
[6]*Endocrinology, Maccabi Health Care Services*

Objective: Primary hyperparathyroidism (PHPT) during pregnancy may pose considerable risks to mother and fetus. This study examined pregnancy outcomes in women with gestational PHPT in relation to clinical and laboratory parameters.

Design: Retrospective case series.

Methods: The study group included 74 women aged 20-40 years who were diagnosed with PHPT following a finding of serum calcium ≥10.5 mg/dl on routine screening at a health maintenance organization (2005-2013) and became pregnant during the time of hypercalcemia (124 pregnancies). Clinical and laboratory data were collected from the files. Pregnancy outcomes were compared with 175 normocalcemic pregnant women (431 pregnancies) tested during the same period.

Results: The cohort represented 0.03% of all women of reproductive age tested for serum calcium during the study period. Abortion occurred in 12/124 pregnancies (9.7%) and other complications in 19 (15.3%), with no statistically significant differences from controls. Hypercalcemia was first detected during pregnancy in 14/74 women (18.9%) and before pregnancy (mean 33.4±29 months) in 60. Serum calcium was measured antenatally in 57/124 pregnancies (46%); mean level was 10.7±0.6 mg/dl (median 10.6 mg/dl). Measurement of serum parathyroid hormone level (with consequent diagnosis of PHPT) was performed during the first studied pregnancy in 17/74 women (23%), before pregnancy (mean 37.8±25.5 months, median 34) in 23 (31.1%), and after delivery (mean 54.7±45.7 months, median 35) in 34 (45.9%). Forty-three women (58.1%) underwent parathyroidectomy, 6 during pregnancy, without maternal or fetal complications. No difference was found in abortion or any pregnancy-related complication between patients who subsequently underwent/did not undergo parathyroidectomy. No significant correlation between calcium level during pregnancy and pregnancy outcomes was found

Conclusions: Serum calcium levels are usually only mildly elevated during pregnancy in women with PHPT. A significant proportion of cases go undiagnosed. Mild hypercalcemia in gestational PHPT is generally not associated with an increased risk of obstetrical complications.

Clinical Characteristics of Male Patients with Osteoporosis Referred To Endocrine Clinic of Single Tertiary Hospital

Gloria Tsvetov[1,2], Ilana Shraga-Slutzky[1,2], Dania Hirsch[1,2], Hiba Masri[1,2], Alexander Gorshtein[1,2], Ilan Shimon[1,2]

[1]*Institute of Endocrinology, Rabin Medical Center, Beilinson Hospital, Israel*

[2]*Sackler Faculty of Medicine, Tel Aviv University*

Osteoporosis in men is underdiagnosed and undertreated. The prevalence of male osteoporosis increases with age, and it becomes a significant public health burden. Currently there are a few clinical studies on male osteoporosis with limited knowledge of effective therapeutic options. The aim of our study was to characterize men with osteoporosis in a referral metabolic clinic. Patients and methods. In this study we retrospectively analyzed medical records of 270 consecutive male patients with osteoporosis diagnosed and treated in our clinic during 2013. **Results:** 270 of 1940 (14%) patients with osteoporosis treated in our clinic were males. The mean age of men with osteoporosis was 67.9 ± 13.6. 113 (40%) men suffered from osteoporotic fractures, 57 of them (50%) had vertebral fractures, 19 (17%) had more than one fracture. Osteoporotic fracture was the first presentation in 82% of men with fractures (age of presentation 62.2 ± 14.5). 141 patients (52%) had vitamin D insufficiency (levels 60 nmol/l, normal 75-250nmol/l), and mean level of 25OHD in these patients was 39.9±12.6 nmol/l. Secondary osteoporosis was identified in 166 (61%) men. The most common etiologies were chronic glucocorticoid treatment (45%), hypogonadism (36%), hypercalciuria (30%) and hyperparathyroidism (19%). Most patients (223) received bisphosphonates as primary therapy, and alendronate was the mostly prescribed agent. **Conclusions:** Osteoporosis in men usually remains an underdiagnosed disorder until an osteoporotic fracture occurs. Secondary causes for osteoporosis are commonly encountered, of which glucocorticoid treatment and hypogonadism are the most prevalent etiologies. Increasing awareness of osteoporosis in men may prevent the deleterious consequences of this disabling but treatable disease.

Compensatory Mechanisms In Mouse Offspring With Inherently Weak Bones Are Suggesting a Gene-by-Environment Interaction In Utero.

Maria Raygorodskaya[1], Arkady Torchinsky[2], Yankel Gabet[2], Chen Shochat[1], David Karasik[1]

[1]*Faculty of Medicine, Bar Ilan University*

[2]*Sackler Medical School, Tel Aviv University*

Establishment of bone structure and functioning begins early in life. Several studies demonstrated that exposure of mouse embryo to adverse environmental factors can detrimentally affect offspring`s bones later in life. We investigated to what extent the genetic differences between mouse strains translate into variable response to teratogen 5-deoxy-2'-cytidine (5-AZA).

Two inbred strains, C3H/HeJ (C3H, with inherently stronger bones) and C57Bl/6J (C57, with weaker bones), were studied. Female mice were injected with diluted 5-AZA or saline (untreated) at day 10 of pregnancy. Five offspring of mice from each 4 strain/sex groups (C3H males, C3H females, C57 males, C57 females) at two ages (3- and 6-month-old) were used. We compared left femora of treated and untreated mice within each group using micro-CT analysis.

In 3-mo-old offspring, treated C57 females demonstrated decrease of bone mineral density (p0.05). Treated females from both strains demonstrated loss of cortical thickness (p0.05). The same trend was found in C57 males. Significant loss of trabecular thickness was observed in treated C3H females (p0.01).

In 6-mo-old offspring, treated C3H males demonstrated loss of cortical thickness (p0.01), had lower trabecular number and higher trabecular separation (p0.05). Treated C57 males and females demonstrated increase of trabecular thickness (p0.05).

Ultimately, this study demonstrates that exposure to a sub-teratogenic dose of 5-AZA during embryonic life affects bone structure in adult mouse offspring. In 3-month-old offspring, teratogenic exposure caused bone loss in both strains. At the age of 6 months, bone loss was observed in the strain with strong bones (C3H), while in the strain with weak bones (C57) there was bone gain which might be an evidence of gene-by-environment interaction. We hypothesize that in mice with inherently weaker bones the effect of adverse intrauterine environment triggered some compensatory mechanisms, which is reflected in thickening of bone trabeculae by the age of 6 months.

Role of Side Effects, Physician Involvement and Patient Perception in Discontinuing or Switching of Oral Bisphosphonates

Rouach Vanessa[1,2], Gez Sharon[1], Yu Jingbo[5], Goldshtein Inbal[1], Goldstein Dalit[1], Ish Shalom Sophia[4], Chodick Gabriel[1,3]

[1]*Epidemiology and Database Research Unit, Maccabi Healthcare Services, Israel*

[2]*Institute of Hypertension, Metabolism, and Endocrinology, Tel Aviv Souraski Medical Center, Israel*

[3]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

[4]*Rappaport Faculty of Medicine, Technion, Israel*

[5]*Global Health Outcomes, Merck & Company, Inc*

Objective: To assess reasons for non-compliance with oral bisphosphonates among osteoporotic women.

Material and Methods: This is a cross-sectional patient survey of osteoporotic women who were at least 55 years old, initiated treatment (index) between 2010-2012 and were non-compliant with first therapy (defined as medication possession ratio70%) or switched therapy within the first year. Survey participants were identified using Maccabi Health Services computerized database. Patients who gave informed consent completed a 20 minute telephonic survey, assessing reasons for discontinuation and switching including physician involvement, side effects, in-convenience and patient perceptions of bone health and bone medications efficacy.

Results: A total of 493 osteoporotic women completed the survey. At the time of the survey (average 3.3 years following therapy initiation) 40% of the patients had discontinued all anti-osteoporotic therapy (mean MPR at interview=19%), 9% remained on initial therapy (continuers, mean MPR at interview=47%) and 51% were using a switched therapy (switchers, mean MPR at interview=62%). Family history, fracture history, socioeconomic status and index drug class and frequency were similar in all groups, but continuers were more likely to be older (mean age of 67±8 vs. 65±8 among switchers and 64±7 among discontinuers) and initiated therapy closer to survey as compared with switchers and discontinuers. The most common reasons for switch or discontinuation of first medication were gastrointestinal complaints (cited by 26.1%) and physician recommendation (19.4%), whereas inconvenience-related reasons and disbelief in the drug's importance played a smaller role (each reported by less than 5% as major reasons). The most common reasons for complete discontinuation of anti-osteoporotic therapy were side effects (cited by 26.9% as a major reason) and physician recommendation (20.0%).

Conclusions: Our findings emphasize the need for new medications with better tolerability profile to improve treatment compliance. Physician involvement in such important therapeutic decisions as drug discontinuation might be sub-optimal.

Comparison of Long-Term Clinical Outcomes of Hypoparathyroid and Normocalcemic Patients after Thyroidectomy.

Ilana Slutzky-Shraga[1,2,3], Gloria Tzvetov[1,2], Alexander Gorshtein[1,2,3], Danya Hirsch[1,2], Carlos Benbassat[1,2,3], Ilan Shimon[1,2]
[1]*Endocrinology, Rabin Medical Center, Israel*
[2]*Faculty of Medicine, Tel Aviv University, Israel*
[3]*Endocrinology, Clalit Health Service*

Background: Permanent hypoparathyroidism may occur in patients following thyroidectomy. Data regarding clinical implications of this rare iatrogenic complication are scarce.

Objective: To evaluate the risks of renal, bone and cardiovascular complications in hypoparathyroid patients in comparison with normocalcemic patients after thyroidectomy.

Patients and methods: 100 consecutive patients diagnosed with permanent postsurgical hypoparathyroidism at a tertiary medical center were identified by charts review. For each patient age- and follow-up matched normocalcemic control was selected from the Rabin Medical Center Thyroid Cancer Registry. All patients had undergone thyroidectomy from January 1975 to October 2011.

Results: The study group included 88% women. Mean age at the last visit was 60.4±15 years, and mean follow-up duration was 14.7± 2.5 years (range, 1-54). Eighty three patients underwent thyroidectomy because of thyroid cancer. Ten hypoparathyroid patients (10 %) had renal stones/calcinosis compared to 4 (4.5 %) control patients. (Odds Ratio 3.3; 95%CI, 1.0-11.2). In both groups the risk of renal stones was significantly associated with urinary calcium level 300 mg/day, but no association was found with the daily dose of calcium supplements and alpha D3. Compared to controls, those with hypopararathyroidism had a nonsignificant trend of less cardiovascular events (3.4 % vs 5.7%, respectively, p=0.2) and diabetes mellitus (11.4 % vs 15.4%, respectively, p=0.1). Hypoparathyroid patients had significantly higher hip bone mineral density, hip T-score and spine T-score (P 0.01).

Conclusion: Compared with the control group, patients with postoperative hypoparathyroidism have higher T-score, higher risk of renal complications and a trend to less cardiovascular diseases and diabetes mellitus. Further study and formal guidelines for chronic management of hypoparathyroidism are needed to reduce the risk of renal complications.

Bone Mineral Density and Vitamin D Status in Israeli IBD Patients: Possible Influencing Factors

Elena Segal[1,3], Razi Evendar[1], Yehuda Hovers[2,3], Yoav Mazor[2], Sophia Ish-Shalom[3]

[1]*Endocrine Institute, Rambam Health Care Campus, Israel*
[2]*Gasroenterology Department, Rambam Health Care Campus, Israel*
[3]*The Ruth & Bruce Rappaport Faculty of Medicine, Technion -The Israel Institute of Technology, Israel*

Background: Inflammatory bowel disease (IBD) patients have decreased bone mineral density (BMD). There is limited consensus regarding the major factors affecting BMD in these patients. Few studies have examined possible genetic factors.

Study Aim: to evaluate bone metabolic status, to determine impact of different factors on BMD in IBD pts.

Patients and Methods: 142 IBD clinic patients (pts) in Rambam Health Care Campus (RHCC). All pts underwent metabolic bone statue evaluation in the Bone metabolism clinic at RHCC: routine blood biochemistry, serum PTH, 25OHD, bone densitometry at the lumbar spine (LS), femoral neck (FN), total hip (TH). Genetic testing for common mutations in NOD2 was conducted.

Results:142 pts, aged 17-79 years; 107 (75.3%) had Crohn's disease (CD): 43 (40.2%) men, 64 (58.8%) women; 35 (24.7%) had ulcerative colitis (UC): 13 (37.1%) men, 23(62.9%) women. 126 (88.7%)- Jewish, 16 (11.3%)- not Jewish.

74 (52%) were treated with glucocorticoids 3 months. 20 (18.7%) of CD pts were treated with anti TNF 3 months.

96 (67.6%) underwent genetic evaluation for NOD2 mutations.

In 60 pts e 25OHD was 20ng/ml, of them 14

22 pts had BMD Z scores-2.5, 18 CD pts, 4 UC pts, 14 men, 8 women. In70 pts

-1 Z-scores -2.5; 51 of CD, 19 of UC, 24 men, 46 women. Higher BMI was associated with increase in Z-score of 0.048 at LS, 0.044 at TH, p=0.04 and 0.02, respectively per BMI unit

In multiple regression analysis mean LS Z-scores were lower in men then in women (CD and UC):-1.71 compared to -1.14, P=0.007.

Anti-TNF treatment was associated with a decrease in TH mean Z-scores: -0.8 versus 1.62, p=0.08.-

Jewish CD patients carrying the mutation SNP20 in the NOD2 gene, had mean Z score -2.49 in TH compared to non-carriers -0.57, P=0.0079.

Conclusion: Lower BMD in IBD patients was associated with male gender, lower BMI, and mutation in NOD2 gene among Jewish CD patients.

"Let's Hold the Bone Together" - Orthopedic-Metabolic Collaborative Management for Osteoporotic Hip Fracture

Meir Frankel[1], Osnat Tel-Levanon[1], Michael Tamar[2], Eyal Yakobi[2], Dan Nabriski[1], Doron Netzer[3], Yaron Brin[2], Meir Niska[2], Pnina Rotman-Pikielny[1]
[1]*Endocrinology Institute, Meir Medical Center, Israel*
[2]*Orthopedic Surgery Departmant, Meir Medical Center, Israel*
[3]*Meir Medical Center, Meir Medical Center, Israel*

Background: Osteoporotic hip fractures are associated with increased morbidity and mortality in older adults. Appropriate metabolic treatment can reduce the risk of future fractures. Only 30% of patients receive calcium and vitamin D supplementation, and 10-15% osteoporosis-specific treatment. Previous research indicated that involvement of a multi-disciplinary professional team can improve the quality of care for hip fracture patients.

Methods: An Orthopedic-Metabolic team was established at a university-affiliated hospital in Israel, for managing hip fracture patients. The intervention included staff educational activities, a structured, in-hospital treatment protocol, and a collaborative follow-up clinic. An observational study evaluated the impact of the intervention.

Results: During 7 months, 222 patients with osteoporotic hip fractures were operated. Thirty died within 6 months of surgery and were excluded. Among the remaining 192 patients, 80% had vitamin D levels tested on the ward (mean 44 nmol/L); over 84% began calcium and vitamin D supplementation; 79% and 47% came to follow-up Orthopedic and Metabolic Clinic, respectively. Of patients that came to the metabolic clinic (mean duration between surgery and metabolic clinic visit - 94 days), 46% started pharmacological therapy at the first visit, while others needed vitamin D loading or additional investigation. Attending a follow-up clinic visit correlated with competent cognitive state, home residency and Clalit HMO (health management organization) membership.

Conclusion: An Orthopedic-Metabolic team can effectively improve quality of care for patients with osteoporotic hip fractures. Yet, only 50% of the patients arrived for a follow-up metabolic visit, and of those, only half received specific treatment recommendations at that visit. Additional research is required to enhance adherence to follow-up visits and treatment decision-making during visits.

Hypernatremia ad Copeptin Levels in the Elderly Hospitalized Patient

Michal Liber[2], Moshe Sonnenblick[2], **Gabriel Munter**[1]
[1]*Internal Medicine and Endocrinology, Shaare Zedek Medical Center Affiliated with the Faculty of Medicine-Hebrew University, Israel*
[2]*Geriatrics Department, Shaare Zedek Medical Center Affiliated with the Faculty of Medicine-Hebrew University, Israel*

Introduction/Objective: Elderly patients have a higher prevalence of hypernatremia. Previous studies suggested that impaired ADH secretion contributes to development of hypernatremia in the elderly. Copeptin is the C terminal part of the ADH precursor and is more stable in plasma than ADH. The aim of this study was to detect demographic and clinical characteristics of the elderly hypernatremic patient hospitalized in the internal medicine ward, and to increase the understanding of the role of ADH secretion in the pathogenesis of hypernatremia.

Design: Case-control study

Setting: Internal Medicine ward in a University affiliated hospital.

Participants: 33 hypernatremic patients (admission sodium150 meq/l, age70) compared to 34 normonatremic patients.

Measurements: Demographic, functional (mental status and ADL) and clinical data (APACHE II score) were collected at admission. Serum Copeptin levels were obtained 48 hours from admission. Mortality and change in the functional status were followed up to 30 days after discharge.

Results: Patients with hypernatremia presented with significantly lower baseline functional and cognitive states and higher APACHE II score (21.3 ±8.6 vs. 15.4 ±6.7, P 0.01). Dementia was present in 97% of the hypernatremic patients compared to 46% of the control group(p0.001). Mortality within 30 days of discharge was higher in the hypernatremic group (58% vs. 32%, P0.05). Higher Copeptin levels were found in the hypernatremic group compared to the normonatremic group (100.2±60.6 pmol/L vs. 66.5±57.2 pmol/l, P0.05). High levels of Copeptin were associated with higher in hospital (P0.05) and 30 days mortality (P0.01). Sodium levels were found to correlate with Copeptin levels; yet, an even stronger correlation was demonstrated between Copeptin levels and Apache II score (r= 0.52, p0.001).

Conclusions: Hypernatremia in the elderly at admission is associated with a high rate of mortality. Copeptin is appropriately secreted by the elderly patient with dementia and seems to be a good single disease severity marker.

Skeletal Effect of Casein and Whey Protein Intake during Catch-Up Growth in Young Male Sprague-Dawley Rats

Majdi Masarwi[1,2], Yankel Gabet[1], Oleg Dolkart[3], Tamer Brosh[6], Biana Shtaif[1,2], Raanan Shamir[1,5], Moshe Phillip[1,2,4], **Galia Gat Yablonski**[1,2,4]

[1]*Sackler School of Medicine, Tel Aviv University, Israel*

[2]*The Laboratory for Molecular Endocrinology and Diabetes, Felsenstein Medical Research Center, Israel*

[3]*Orthopedic Surgery Division, Tel Aviv Medical Center, Israel*

[4]*Endocrinology and Diabetes, Schneider Children's Medical Center, Israel*

[5]*Institute for Gastroenterology, Schneider Children's Medical Center, Israel*

[6]*School of Dental Medicine, Tel Aviv University, Israel*

The ratio of casein to whey, the two most prevalent milk proteins, differs between human milk and cow milk used in infant formulas. The aim of the present study was to determine if the type of milk protein ingested influences catch up (CU) growth. Young male Sprague-Dawley rats were either fed *ad libitum* (controls) or subjected to 36 days of 40% food restriction followed by short (24 days) or long (40 days) periods of re-feeding with an iso-caloric, iso-protein diet containing either a vegetarian source of protein (standard rat chow) or an animal source of protein (milk proteins casein or whey). In terms of body weight, catch-up growth was incomplete in all study groups. Despite their similar food consumption, casein-re-fed rats had a significantly higher body weight and longer humerus than whey-re-fed rats in the long-term. Both casein and whey groups had a higher epiphyseal growth plate (EGP) than the rats re-fed normal chow. Micro-computed tomography (μCT) yielded significant differences in bone microstructure between the casein and whey groups, with the casein-re-fed animals having greater cortical thickness in both the short and long term in addition to a higher trabecular bone fraction in the short term, although this difference disappeared in the long term. Mechanical testing confirmed the greater bone strength in the rats re-fed casein. In conclusion, bone quality during CU growth significantly depends on the type of protein ingested. The higher EGP in the casein- and whey-re-fed rats relative to the rats re-fed regular chow suggests a better growth potential of milk (animal protein)-based diets. The results of our study suggest that whey may lead to slower bone growth with reduced weight gain and as such, may serve to circumvent long-term complications of CU growth.

Growth and Ponderosity Determine the Infancy to Childhood Transition

Alina German[1], Gregory Livshits[2], Inga Peter[3], Ze'ev Hochberg[4]
[1]*Pediatric Department, Clalit Health Service, Israel*
[2]*Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Israel*
[3]*Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, USA*
[4]*Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Israel*

Context: The transition from infancy to childhood at age 7-13 months is marked by a growth spurt, when the GH-IGF-1 axis activity sets in, and determines ~50% of the variation in adult height. We recently showed that the variance provided to adult height by the ICT is adaptive to the environment, with 42% contribution by intrauterine and social cues, with no detectable genetic involvement (J Peds 2015). AIM: We aimed to identify the remaining 58% signal for ICT, and hypothesized that it is related to auxological parameters.

Methods: The study examined growth patterns and identified the ICT in 185 well-baby clinic boys(M) and 200 girls(F). Curve fitting was used to examine continuous growth from a set of discrete measurements of length(L) and weight(W), and we calculated the ponderal index(PI). A linear general regression model was used to assess the correlation between ICT age and pre-ICT L- and W-velocity (LV, WV) and PI at ICT.

Results: In M, the ICT (at age 10.5±1.7m, m±SD) occurred at L 72.6±2.9cm, W 9.1±0.9 kg, BMI 17.2±1.3 kg/m², after pre-ICT WV of 0.38±0.12 kg/m and LV of 1.5±0.46 cm/m. In F, the ICT (at age 9.7±1.7 m, pvs. M) occurred at L 72±3,0 cm (p0.0001), W 8.3±1.0 (p0.0001), BMI 16.8±1.4 (p=0.004), after WV of 0.41±0.11kg/m (p=0.01) and LV of 1.6±0.36 cm/m (p=0.03). The PI was similar among M (23.7±2.1) and F (23.9±2.4 kg/m^3). A linear general regression model adjusted for gestation age, discovered a linear correlation between the ICT age and pre-ICT LV (p0.0001), PI (p0.0001) and sex (p0.001), which explained 62% of the variation in ICT age.

Conclusions: The plasticity in adult height, as determined by the ICT (age of GH-IGF1 setting in), is set-in mostly by pre-ICT length velocity and ponderal index (~60%), as well as by social cues and the intrauterine environment (~40%).

Histone Deacetylase Inhibition Markedly Enhances the Efficacy of Chemo-Radiation Treatment in Human Non-Small Lung Cancer Cells

Vladimir Gavrilov[1], Konstantin Lavrenkov[2], Shmuel Ariad[2], Shraga Shany[3]
[1]*Clinical Biochemistry and Phamacology, Ben Gurion University, Israel*
[2]*Oncology, Ben Gurion University, Israel*
[3]*Clinical Biochemistry and Pharmacology, Ben Gurion University, Israel*

Background: Lung cancer is the leading cause of cancer-related mortality. Non-small cell lung cancer (NSCLC) accounts for 80%–85% of all human lung cancer cases. A considerable number (30%) of NSCLC patients present with locally advanced, un-resectable stage III disease. These patients are treated by combinations of chemo- and radiotherapy. This treatment includes Vinorelbine (VIN), a vinca alkaloid, in association with the platinum drug cisplatin (CIS) and ionizing radiation (IR). However, therapy outcomes are poor. In order to enhance the anticancer activity, sodium valproate (VPA), a histone deacetylase (HDAC) inhibitor, was added to the conventional treatment protocol, in the present study. We hypothesize that the addition of a HDAC inhibitor to the VIN-CIS-IR combination would enhance the efficacy of the treatment by impairing the repair ability of cancer cells.

Aim: To evaluate a putative enhanced anticancer effect on the human NSCLC A549 cell line. following addition of VPA to the combined CIS-VIN-IR treatment.

Methods: NSCLC A549 cancer cells were treated with CIS, VIN, VPA and IR alone or in combinations. Cell proliferation, cell-cycle, apoptosis, levels of DNA double-strand breaks (DSBs), activated DNA damage checkpoint kinases pChk1, pChk2, were tested.

Results: A combination of CIS (0.2 μg/ml), VIN (2 nM) and IR (2.5 Gy) inhibited A549 cell proliferation by 34.8%. However, addition of VPA (1 mM) decreased cancer cell proliferation by 48.8% (p0.0001). The four-agents combination exhibited the highest DNA damaging effect and increased the expression of DSBs by 91.6% (p0.0001). The treatment markedly increased (p0.001) levels of pChk2, pChk1, p21Cip1/Waf1 and p27Kip1. These extensive molecular changes led to cell-cycle stalling and apoptosis.

Conclusions: Inhibition of histone deacetylase activity by VPA, markedly enhances the anticancer activity of the conventional CIS-VIN-IR combination. This finding may have translational implications in providing a novel NSCLC treatment protocol for enhancing the efficacy of anticancer treatment and possibly for attenuating severe drugs and radiation provoked side effects, by reducing their doses.

Cardiovascular Risk Assessment And Carotid Intima-Media Thickness In Young Adults, Survivors Of Hodgkin`s Lymphoma

Hofit Cohen[1,4], Rafael Bitzur[1,4], Michael Shechter[2,4], Dror Harats[1,4], Dalit Modan[3,4]

[1]*The Bert W. Strassburger Lipid Center, The Chaim Sheba Medical Center, Israel*
[2]*Clinical Research Unit, The Chaim Sheba Medical Center, Israel*
[3]*Pediatric Endocrinology and Diabetes Unit, Safra Children's Hospital, the Chaim Sheba Medical Center, Israel*
[4]*Sackler Faculty of Medicine, Tel-Aviv University, Israel*

Cardiovascular disease (CVD) is a common non-malignant cause of death in Hodgkin`s lymphoma (HL) survivors.

We aimed to perform a wide cardiovascular risk assessment in survivors of childhood and adolescence HL and hypothesized that survivors will have an altered increased rate of endothelial function compared with healthy peers.

27 patients (females=14; aged 25±5y, 8.6±4 years after diagnosis) were recruited. 17 were treated with chemotherapy and chest irradiation, nine with chemotherapy alone, and one with radiation only. All patients were euthyroid at the time of evaluation. Cardiovascular risk assessment included brachial artery flow-mediated dilation (FMD), IMT measurement with echo-color Doppler, an extended lipid profile, liver function tests, glucose and insulin levels, HS-CRP and lifestyle assessment. Control group for IMT measurements comprised 55 (33 females) healthy normo-cholesterolemic patients aged 18-30 years.

Results: Risk factors: 5 patients were smokers, 11 patients had a family history of CVD, and none had hypertension or diabetes. Mean BMI was 23.9±4.2kg/m2, waist circumference of males 84.9±9.4cm and of females=79.1±8.7cm. 6/27 had increased insulin resistance as calculated in the Homeostasis Model Assessment (HOMA).Mean cholesterol levels =179.8±37 mg/dl; mean LDL-c =111±35.8mg/dl, mean TG =107-±52.8 mg/dl, and mean HDL-c levels =53.2±15.2mg/dl. Lp (a) levels were elevated in 6 patients. Apo A1 levels =134.3±25.2 mg/dl and Apo B levels = 88.3 ±23.7, mean HS-CRP levels =4.61±6.9.One participant fulfilled the criteria for the metabolic syndrome and four others fulfilled 2 out of 5 criteria. Five patients had abnormal endothelial function.

Average IMT was 0.537±0.11mm, compared to 0.49±0.056mm in controls and four patients had increased carotid IMT according to current normal range (0.65mm for

Conclusions: Our results suggest the presence of pro-atherogenic markers and metabolic abnormalities in these young patients, which may enhance their risk for premature atherosclerosis and requires further investigation and prevention measures.

Basal Luteinizing Hormone Combined with the Tanner Stage of Breast Development Can Predict the Gonadotropin Response Following Gonadotropin-Releasing Hormone Stimulation Test

Osnat Admoni[1], Naama Schwartz[2], Yardena Tenenbaum-Rakover[1]
[1]*Pediatric Endocrine Unit, Ha'Emek Medical Center, Israel*
[2]*Clinical Research Unit, Ha'Emek Medical Center, Israel*

Background: The gonadotropin-releasing hormone stimulation test is widely used in the evaluation of precocious puberty but it requires repeated blood sampling, time-consuming and uncomfortable for the patients.

Objective and hypotheses: To identify which parameters can be used to predict pubertal response in GnRH stimulating test for the diagnosis of central precocious puberty (CPP) in girls.

Method: Included in the study 123 girls aged 3-9 years that underwent GnRH stimulating test due to early pubertal development. Clinical parameters were collected from the medical files. The results of GnRH tests were retrieved retrospectively. Stepwise logistic regression was used to identify which variables can predict the probability for GnRH pubertal response (Peak LH ≥6 IU/L).

Results: Included in the final model the following variables; age, Tanner breast stage (B1-5) and basal LH with cut of point of LH 0.3 IU/L (*P* 0.0001, C- statistic 84.3%). In girls aged 3-6 years that presented with Tanner B2 and in girls aged 7-9 years that presented with Tanner B1, when basal LH was ≤0.3 IU/L, the predictive probability for pubertal response in GnRH test was less than 9%.

In girls aged 3-9 years that presented with Tanner B3 and with basal LH higher than 0.3 IU/L, the probability for pubertal response was more than 72%. BMI and advanced bone age were not significantly associated with GnRH pubertal response

Conclusion: Basal LH combined with age and Tanner breast stage can predict the response to GnRH test, therefore GnRH test is unnecessary in the majority of the cases for the diagnosis of CPP in girls.

Modulation of Rat Behaviors and the Stress Response by Duration of the Infancy Stage

Yonatan Crispel, Oren Katz, Inna Kogan, Zeev Hochberg
Rappaport Family Faculty of Medicine, Technion , Haifa, Israel

The age at weaning programs life history adaptively. Shorter infancy resulted in longer/thinner animals with a reproductive-strategic shift to earlier physical and sexual development (BMC Med, 2013). HYPOTHESIS: The length of infancy impacts also the stress-response and has behavioral consequences. METHODS: Sprague-Dawley pups (generation F1), which usually are weaned at age 21 days, were weaned by cross-fostering at age 16, 21 or 26 days, and separated from their foster mothers at d30. At d60 females (F) and males (M) were mated within the weaning groups and generation F1, F2 and F3 animals were tested in an open field maze weekly from age 22-60 days for motor activity, anxiety/curiosity, short-term memory and stress-related corticosterone (CS) levels. RESULTS: Motor activity in an open field was similar in the three groups at generation F1, but increase in d16 vs. d26 animals by a mean19% in F and 27% in M rats at F2 (p0.05) and 16% (F and M) in F3 (p0.02). Anxiety, measured as time spent next to the maze walls, was similar at F1, but smaller by 58% and 68% in d16 vs. d26 young animals at F2 (p0.05) and F3 (p0.05). Curiosity was greater in d16- F2 and F3 rats *vs* d26-weaned rats (p0.05). Open-field anxiety was associated with greater CS levels in d16-weaned (25.3 ± 4.6 mcg%) as compared to d26-weaned animals (11.6 ± 0.8 mcg%, p0.01). Short-term memory, measured as object recognition, was similar between groups in F1 but better in d16 F2 as compared to d26-weaned rats by a mean 25% in M and 19% in F (p0.05). The difference was 10% in both F and M at F3. CONCLUSIONS: The age at weaning programs the stress-response and behaviors adaptively. In line with a faster reproductive strategy, shorter infancy resulted in a shift to greater motor activity, short-term memory and curiosity, and smaller anxiety but greater associated CS response. M were affected more than F animals and these traits built up trans-generations.

Young Ethiopian Immigrants Are At Greater Risk For Diabetes And Are BMI Sensitive As Compared To New Immigrants From The Former Soviet Union [FSUI]

Anat Jaffe[1], Shmuel M Giveon[3], Liat Wulffhart[2], Bernice Oberman[2], Laurence Freedman[2], Arnona Ziv[2], Ofra Kalter-Leibovici[2]
[1]*Endocrinology, Hille Yaffe Medical Center, Israel*
[2]*Cardiocascular, Gertner Institue for Epidemiology & Health Policy, Israel*
[3]*Family Medicine, Clalit Health Services, Israel*

Background: We previously reported that diabetes risk in young (age 20-49 years) Ethiopian-Jewish-Immigrants [EJI] is higher than among non-Ethiopian-Jewish subjects (NEJ). However, immigration stress may affect diabetes risk, thus masking the role of ethnicity. We therefore compared the risk of diabetes in EJI to the risk among former-Soviet-Union immigrants (FSUI), in order to distinguish between the effect of ethnicity and the effect of immigration stress.

Objective To calculate diabetes risk among young EJI and FSUI compared to born-in-Israel-NEJ (bNEJ) and determine the associations between BMI and incident diabetes in each population group.

Methods: Data was retrieved from the computerized database of the Sharon-Shomron region of Clalit Health Services for 5,638 EJI and matched controls NEJ for the years 2007-2011. The controls included 9,172-bNEJ and 1,106-FSUI subjects. bNEJ served as the comparison group. Sex-adjusted hazard ratios [HRs] for incident diabetes were calculated. The difference in BMI between those diagnosed with diabetes and non-diabetics was determined within each ethnic group.

Results: The sex-adjusted HR for incident diabetes in young FSUI was similar to the bNEJ. However, EJI had a greater diabetes risk; sex-adjusted HR- 1.8 (95% CI: 1.5, 2.2). Non-diabetic EJI had significantly lower BMI than non-diabetic subjects from the FSUI and bNEJ (p0.0001). The mean difference in BMI between people, who developed diabetes during follow-up, and those who remained non-diabetic differed by ethnicity. bNEJ who developed diabetes had a 5.3 kg/m^2 higher BMI than their corresponding non-diabetic group, whereas for EJI the difference was only 3.0 kg/m^2. The mean baseline BMI of EJI who developed diabetes during follow-up was 25.9 kg/m^2, similar to the baseline BMI of the non-diabetic bNEJ and FSUI participants.

Conclusions: Young Ethiopian immigrants are at greater risk for diabetes compared to age-matched FSUI and bNEJ subjects, and develop diabetes at lower BMI levels. A modified definition for adequate BMI in EJI is needed.

Estradiol-17β Induced Pancreatic Beta Cell Proliferation is Foxo1 Dependent

Meital Grafi-Cohen[1], Dalia Somjen[1], Orly Sharon[1], Nadav Sagiv[1], Rona Limor[1], **Sigal Shaklai**[1], Naftali Stern[1,2]

[1]*Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center*

[2]*Sackler Faculty of Medicine, Tel-Aviv University*

The Foxo1 transcription factor plays a major role in β-cell proliferation, differentiation and protection from oxidative stress. Foxo1 activity is regulated by phosphorylation, which leads to cytoplasmic translocation and inactivation. Interaction of Foxo1 with estrogen receptor α (ER) was has been shown to influence transcription and cell cycle in estrogen responsive tissues. Human and rodent β-cells express the three ERs; ERα, ERβ and the G-protein coupled ER (GPER). Here, we examine the effect of estradiol-17β (E2) as mediated by the three ER subtypes on β-cell proliferation in conditions of normo- and hyperglycemia. We also examine the contribution of Foxo1 to E2-induced β-cell proliferation by knocking down (KD) its expression using siRNA.

The effect of E2 on proliferation was assessed in INS1 (rat) and MIN6 (mouse) insulinoma cells by thymidine incorporation. Under normal growth conditions, E2 and either ERα, ERβ or GPER-specific agonists enhanced proliferation (~3Xthree folds). Upon exposure to 24h of hyperglycemia, however, only the ERα agonist PPT retained the ability to induce proliferation. Prolonged (5 days) hyperglycemia restored the proliferative effect of E2 and all ER-specific agonists. Concordantly, 24 hours exposure to high glucose resulted in a significant reduction of ERβ and GPER mRNA and protein expression, with subsequent increase at day 5. Expression of ERα remained stable. Foxo1 knockdown by siRNA completely abrogated the proliferative effects of E2 and ER agonists. E2 treatment elicited a significant reduction of Foxo1 within 30 minutes, which returned to baseline at 24h. The decline in Foxo1 occured primarily in the cytoplasmic compartment and was not prevented by the ubiquitin inhibitor MG132. pAKT levels mirrored those of Foxo1 while pFoxo1 increased six fold during the entire 24h of exposure to E2.

Our findings support a novel co-operation between E2 and Foxo1 in β-cells and suggest that E2-induced β-cell proliferation is mediated through Foxo1.

Adult Israeli Arabs have a High Incidence of Diabetes Mellitus in all Sex and Age Sub-Groups

Anat Jaffe[1], Shmuel M Giveon[2], Liat Wulffhart[3], Bernice Oberman[3], Arnona Ziv[3], Laurence Freedman[3], Ofra Kalter-Leibovici[3]

[1]*Endocrinology, Hille Yaffe Medical Center, Israel*

[2]*Family Medicine, Clalit Health Services*

[3]*Cardiocascular, Gertner Institue for Epidemiology & Health Policy, Israel*

Background: Diabetes mellitus is an emerging epidemic in the Arab world. In Israel diabetes prevalence (not age-adjusted) in adult Arabs is higher than among adult Jews: 21% versus 12%.

Objective: To determine sex and age specific diabetes incidence, prevalence and diabetes risk, adjusted for certain components of the metabolic syndrome (MetS) among Arabs and Jews in Israel.

Methods: The computerized database of the Clalit Health Services for the Sharon-Shomron region between 2007 and 2011 was searched for Arab (mostly Muslim) and non-Ethiopian Jewish subjects, born before 1.1.1988 and who survived until at least 1.1.2009. The groups were matched for age and sex. The prevalence in 2007, the weighted incidence examined over the 4-year period (2008-2011) and hazard ratios [HRs] adjusted for sex and MetS components were calculated for both groups.

Results: 17,044 Arabs (49% males) and 16,012 Jews (50% males) were included. Age-adjusted diabetes incidence [%; 95% confidence interval (CI)] was significantly higher among Arabs [2.9 (2.7, 3.1)] than among Jews [1.7 (1.6, 1.8)]. This held true across all age- and sex subgroups. Adjustments for MetS components, except BMI, did not significantly change the HR estimates. Adjustment for BMI attenuated the association between ethnicity and diabetes risk but it remained highly significant. Diabetes prevalence was 18.4% among Arabs and 10.3% among Jews. The prevalence (95%CI) among Arab females was somewhat higher than in Arab-males; 20.03% (19, 21) versus 16.7% (15.7, 17.8), respectively.

Conclusions: Arabs in Israel have high diabetes prevalence and incidence. The excess risk for diabetes in Arabs appears in all age groups and is partially explained by the high prevalence of obesity.

Clinical and Genetic Characteristics and Therapeutic Challenges in an Infant Born with Severe SGA and Neonatal Diabetes

Adi Auerbach[1], Noa Ofek Shlomai[2], Ariella Weinberg Shokrun[3], Ephrat Levy-Lahad[3], David Zangen[1]

[1]*Division of Pediatric Endocrinology, Hadassah Hebrew University Medical Center, Israel*

[2]*Department of Neonatology, Hadassah Hebrew University Medical Center, Israel*

[3]*Department of Genetics, Shaare Zedek Medical Center, Israel*

Background: Neonatal Diabetes Mellitus (NDM), a rare genetic condition, mostly caused by mutations in genes encoding the beta-cell-K_{ATP}-channel, is characterized by hyperglycemia requiring insulin therapy in the first 6 months of life.

Recent studies indicate that within consanguineous Arab populations the commonest etiologies for NDM differ from the ones in Caucasians. In cases of beta-cell-K_{ATP}-channel mutation sulfonylurea may replace the difficult neonatal insulin dependency.

Objective: Characterizing the significant therapeutic challenges and the genotype of an infant born with extremely low birth weight (1.080 Kg) with early severe NDM to consanguineous Palestinian parents.

Methods and Results: Homozygosity mapping revealed that 3 genes (INS, SLC19A2 , RFX6) causing NDM, are included within large homozygous segments in the patient's DNA. Insulin gene sequencing was normal. Extended sequences of other candidate genes is currently performed.

Therapeutically, the thin sub-dermal fat tissue limited the use of insulin pump or continuous glucose monitoring. In order to optimize the treatment, we conducted a therapeutic trial by sulphonylurea prior to genetic diagnosis although the probability of K_{ATP}-channel mutation was low in this case. This treatment was unhelpful, reinforcing the search for other genetic causes.

Despite a previous report suggesting the superiority of Glargine over Detemir in controlling blood glucose levels in extremely low birth weight infants, in our case the response to both of them was similar and 3 daily doses of Long Acting Insulin Analogues (LAIA) were required to achieve optimal glycemic control.

Conclusion: NDM in low birth weight infants is a difficult therapeutic challenge. A genetic diagnosis is mandatory but empiric trial of oral hypoglycemic agents may be beneficial and should not be delayed. If insulin is required LAIA in few daily doses are the optimal choice.

Inhibition of Glucose Damage to Endothelial Cells by Yeast Derived Anti-Diabetic Substance

Nitsa Mirsky, Yulia Schnieder, Galit Fadida
Human Biology, University of Haifa, Israel

Vascular diseases are a severe complication of diabetes, leading to CVD, strokes, nephropathy, and retinopathy. Endothelial dysfunction is the initiating event of atherosclerosis, comprising of impaired vasodilatation, inflammation, and reduced NO production. Oxidative stress plays a key role in this process. Addition of insulin protects the cells from high glucose damages.

The aim of our study was to investigate the mechanism of high glucose damage to endothelial cells, and to examine the effects of Glucose Tolerance Factor (GTF), an anti diabetic agent extracted from yeast, on these parameters.

Previously we found that treatment with GTF decreased blood glucose and lipids in diabetic animals. In vitro studies showed that GTF phoshorylated key proteins along insulin pathway.

In the present study we found that high glucose decreased ABAE endothelial cells proliferation to 83.9% and 60.2% (for x5 and x10 glucose concentrations, respectively). Addition of GTF (5mg/ml) increased the proliferation to 98.9%, and 75.4% (respectively).

High glucose (x10), impaired catalase and SOD activity to 83.7% and 72.3% respectively. Addition of GTF (1mg/ml) to high glucose (x10) conditions, increased catalase and SOD activities to 100.5% and 92% respectively. Addition of GTF (1mg/ml or 5mg/ml) reversed NO production decreased by high glucose, to 84.1% or 89.2%, respectively.

Addition of GTF increased key proteins (AKT, MAPK. PTEN, eNOS) phosphorylation along insulin signaling pathway. GTF effect was dose and time dependent and in both normal and high glucose conditions.

Our findings present GTF as a novel insulin-like material that can protect endothelial cells from high glucose damages.

Pediatric Wolfram Syndrome Misdiagnosed as Type 1 Diabetes Mellitus

Ilana Koren, Pnina Vardi
Pediatric Diabetes Clinic Armon Child Center, Clalit Health Services, Israel

Introduction: Wolfram syndrome 1 (WS1) is a rare (1/770,000 in the United Kingdom) autosomal recessive disorder characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DIDMOAD syndrome). It is caused by a mutation in the *WFS1* gene (chromosome 4p16.1) which encodes wolframin (a transmembrane protein of pancreatic β cells). Insulin-dependent diabetes mellitus develops at an average age of 6 years, optic atrophy at an average age of 11 and partial central diabetes insipidus and deafness in adolescence. Additional manifestations include neurologic abnormalities (truncal ataxia, myoclonus, epilepsy, nystagmus, and hyposmia), renal tract abnormalities, psychiatric manifestations and gonadal disorders.

Aim: To assess retrospectively WS1 patients who were originally misdiagnosed as type 1 diabetes mellitus.

Methods: Medical histories of 6 patients with clinical misdiagnosis of type 1 diabetes later diagnosed with WS1 were reviewed.

Results: All 6 WS1 patients aged 6-12 years at diagnosis were misdiagnosed as type 1 diabetes mellitus. All 6 patients had unusual symptoms which accompanied diabetes at diagnosis (including urine and fecal incontinence, optic atrophy, deafness and psychiatric manifestations) ,unlike the known literature.

Conclusions: Cases of Wolfram syndrome 1 may remain undiagnosed due to misdiagnosis as type 1 diabetes mellitus and incorrect interpretation of clinical symptoms of neurodegenerative abnormalities, especially in their early stages. Careful history and physical examination may reveal this entity is not as rare as considered.

Increased Risk of Severe Diabetic Ketoacidosis among Jewish Ultra-orthodox Children

Noah Gruber[1,2], Brian Reichman[2,3], Dalit Modan[1,2], Yonatan Yeshayahu[1,2], Michal Ben-Ami[1,2], Kineret Mazor-Aronovitch[1,2], Yael Levy-Shraga[1,2], Liat Lerner-Geva[2,3], Orit Pinhas-Hamiel[1,2]

[1]*Pediatric Endocrine and Diabetes Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Israel*

[2]*Sackler School of Medicine, Tel-Aviv University, Israel*

[3]*The Women and Children's Health Research Unit, Gertner Institute, Tel Hashomer, Israel*

Objective: Diabetic ketoacidosis (DKA) at diabetes diagnosis is a dangerous yet potentially preventable condition. Young age, low socioeconomic status, and low parental education have been found to be associated with increased risk of DKA. We compared the rate of DKA at type 1 diabetes mellitus diagnosis among ultra-orthodox and secular Jewish children.

Research Design and Methods: An analysis of medical records of all consecutive patients with new-onset type 1 diabetes mellitus who were admitted to one tertiary medical center from January 2007 to January 2014. DKA was defined as venous pH 3^{-} 3^{-}

Results: Of 81 patients with new-onset type 1 diabetes mellitus (38 females, mean±SD age at diagnosis 9.9±4.2 years), 34 (42%) presented with DKA: 21/60 (35%) of patients from secular families and 13/21 (62%) from ultra-orthodox families. Children from ultra-orthodox families had a 3.5-fold increased risk of presenting with DKA than children from secular families (95% CI 1.2-10.1, p= 0.02), and a 3.8 fold risk to be admitted with severe DKA (95% CI 1.1-12.6, p= 0.02). Other factors that were found to be associated with an increased risk of DKA were younger age, an absence of maternal academic education, and residence in an area of low socioeconomic status.

Conclusions: DKA and severe DKA at diabetes diagnosis were more common among religious ultra-orthodox than secular Jewish children. Both increased public awareness and greater medical alertness are necessary to reduce the high rates of DKA in new-onset type 1 diabetes mellitus.

Irisin and the Metabolic Phenotype of Adults with Prader-Willi Syndrome

Harry Hirsch[1], Itai Gross[2], Yehuda Pollak[1,3], Talia Eldar-Geva[4,5], Varda Gross-Tsur[1,5]

[1]*Israel Multidisciplinary Prader-Willi Syndrome Clinic, Neuropediatric Unit, Department of Pediatrics, Shaare Zedek Medical Center, Israel*
[2]*Department of Pediatrics, Hadassah Hospital, Ein Kerem, Israel*
[3]*The School of Education, The Hebrew University, Israel*
[4]*Reproductive Endocrinology and Genetics Unit, Department of Obstetrics and Gynecology, Shaare Zedek Medical Center, Israel*
[5]*The Faculty of Medicine, The Hebrew University, Israel*

Context: Hyperphagia, low resting energy expenditure, and abnormal body composition contribute to severe obesity in Prader Willi syndrome (PWS). Irisin, a circulating myokine, stimulates "browning" of white adipose tissue resulting in increased energy expenditure and improved insulin sensitivity. Irisin has not been previously studied in PWS.

Objectives: Compare plasma and salivary irisin in PWS adults and normal controls. Examine the relationship of irisin to insulin sensitivity and plasma lipids.

Design and Study Participants: A fasting blood sample for glucose, lipids, insulin, leptin, adinopectin, and irisin was obtained from 22 PWS adults and 54 healthy BMI-matched volunteers. Saliva was collected for irisin assay in PWS and controls.

Results: Fasting glucose (77±9 vs 83±7mg/dl, p=0.004), insulin (4.1±2.0 vs 7.9±4.7µU/ml, p0.001), and triglycerides (74±34 vs 109±71mg/dl, p=0.007) were lower in PWS than in controls. Insulin resistance (HOMA-IR) was lower (0.79±0.041 vs 1.63±1.02, p0.001) and insulin sensitivity (QUICKI) was higher (0.41±0.04 vs 0.36±0.03, p0.001) in PWS. Plasma irisin was similar in both groups, but salivary irisin (64.5±52.0 vs 33.0±12.1ng/ml), plasma leptin (33.5±24.2 vs 19.7±19.3ng/ml) and plasma adinopectin (13.0±10.8 vs 7.6±4.5µg/ml) were significantly greater in PWS (p0.001). In PWS, plasma irisin showed positive Pearson correlations with total cholesterol (r=0.58, p=0.005), LDL-cholesterol (r=0.59, p=0.004), and leptin (r=0.43, p=0.045). Salivary irisin correlated negatively with HDL-cholesterol (r=-0.50, p=0.043) and positively with LDL-cholesterol (r=0.51, p=0.037) and triglycerides (r=0.50, p=0.041).

Conclusions: Salivary irisin was markedly elevated in PWS although plasma irisin was similar to levels in controls. Significant associations with plasma lipids suggest that irisin may contribute to the metabolic phenotype of PWS.

The Involvement of Hypothalamic MicroRNA Molecule in the Individual Differences in Response to Weight Cycling

Yonat Drori[1,3], Yael Kuperman[2], Mariana Schroeder[1,3], Meira Weiss, Meira Weiss[1,3], Alon Chen[1,3], Alon Chen

[1]*Department of Neurobiology, Weizmann Institute of Science, Israel*

[2]*Department of Veterinary Resources, Weizmann Institute of Science, Israel*

[3]*Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Germany*

Dieting is the common approach when dealing with overweight and obesity. Unfortunately, the success rate of this method is low. Consumption of a low calorie diet in an attempt to lose weight is generally effective at first, but then, a relapse occurs as a result of "succumbing" to the high fat diet once again. A pattern of repeated weight loss and regain is observed in the obese population and is referred to as weight cycling or "yo-yo dieting". Due to individual differences, the response to metabolic challenges, including alternating consumption of high and low fat diets, varies greatly between humans, yet the mechanisms underlying this response are not fully understood. To date, few studies have been done on weight cycling in rodents, and none of them addressed the issue of individual genetic differences resulting in a different response to weight cycling. We established a weight cycling model in wild type (WT) c57/bl6 mice, consisting of alternating diets of regular chow and high fat (60% of calories) diet. Following 3 cycles of high fat diet / chow, the mice metabolic phenotype was assessed. More than one month following the end of the protocol, it was apparent that not all of the mice showed the same body weight pattern. We categorized the mice into 3 categories: **same** – mice that kept the same body weight, **gained** – mice that gained more weight and **lost** – mice that lost weight. Mice in the 3 groups differed not only in their weight, but also in their metabolic phenotype. Genetic profile of the mice hypothalami revealed a specific microRNA (miR) with significantly different expression level in **gained** versus **lost** mice. We are currently conducting a follow-up study in which we exogenously, using a viral approach, alter this miR expression level in the hypothalamus of WT mice undergoing the weight cycling protocol.

Predictors of Successful Weight Reduction and Maintenance in Obese Children and Adolescents

Shlomit Shalitin[1,2], Moshe Phillip[1,2], Sari Krepel-Volski[3]
[1]*The Jesse Z and Lea Shafer Institute of Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel, Israel*
[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*
[3]*Pediatric Department A, Schneider Children's Medical Center of Israel, Israel*

Objective: To date, efforts to identify predictors of positive response to lifestyle interventions aimed at reducing obesity in children have been inconclusive.

The aim of this study was to explore which baseline variables predict successful weight reduction in obese children and adolescents.

Methods: A retrospective chart review of 286 obese patients (175 girls) followed at a tertiary pediatric obesity clinic was used. Data obtained from the medical records included: demographic and anthropometric characteristics of the patients and their parents. A multivariant regression model examined the effect of potential predictive variables.

Results: Participants were predominantly females (61.2%), 61.3% of them were pre-pubertal with a mean age of 9.4 ± 3.2 years, and a mean BMI -SDS of 2.07 ± 0.56 on presentation. Obesity related comorbidities were present in 31.5% of the cohort. The median duration of follow-up in the clinic was 2.2 years (mean: 2.4±1.2 years).

A reduction in BMI -SDS≥0.2 during the follow-up period was achieved in 40.1% of the patients in the cohort and they were classified as responders.

Baseline characteristics that were significantly different between the responders and non-responders at first visit were: younger age (p=0.0003), higher BMI-SDS (p=0.009), absence of acanthosis nigricans (p=0.01), and a higher maternal educational level (p=0.02). On logistic regression analysis, baseline variables associated with successful weight reduction included: higher BMI-SDS (OR= 2.52; 95% CI 1.36-4.66, p=0.004) and absence of acanthosis nigricans (OR= 2.9; 95% CI 1.35-6.21, p=0.003).

Conclusions: Early intervention in obesity treatment in childhood improves likelihood of success. Other factors such as degree of insulin resistance (expressed by the presence of acanthosis nigricans), higher degree of obesity, and higher maternal educational level also appear to play a role. Assessing characteristics associated with treatment outcome may allow clinicians to individualize a weight management program.

Hepatic Steatosis in Zebrafish (Danio rerio) May Be A Consequence Of Normal Aging

Fadwa Dabbah Assadi, David Karasik, Chen Shochat, Dalia David
Faculty of Medicine, Bar Ilan University, Israel

Bar Ilan University, Faculty of Medicine in the Galilee, Safed, Israel.

We study nonalcoholic fatty liver disease (NAFLD) in small fish called zebrafish (*Danio rerio*), since it is an important vertebrate model for research of human physiology. NAFLD is a common multifactorial disorder. Previous studies showed that the prevalence of NAFLD in humans increases with age. This indicates that this disease may be attributed to age.

In zebrafish, a NAFLD-like syndrome can be modeled by overfeeding of "young adult" fish (diet-induced obesity (DIO) model). Surprisingly, NAFLD was observed by us in aged wild-type zebrafish during a random histological study. Lipid accumulation in liver was observed in 14 mpf (months post fertilization)-old zebrafish raised on a standard diet and in standard environment in our lab. Lipid accumulation was detected by performing lipid staining (using Oil Red O) to visualize intrahepatic localization of lipids within liver tissue sections. We also randomly obtained 14 mpf fish from other labs in Israel and assessed their liver sections.

Lipid accumulation (defined by intrahepatic localization of lipids within liver sections) was measured by ImageJ program. In wild type 14 mpf fish we found higher percent of lipid accumulation (similar to that of 6 mpf DIO fish) compared to 6 mpf fish maintained on a standard diet.

We further tested whether a calorically-restricted nutrition combined with exercise in power-swimming machine, would inhibit lipid accumulation and decrease lipid prevalence in the liver of 14 mpf-old zebrafish. We detected that the percent of lipid accumulation was reduced in fish thusly treated.

In conclusion, our findings indicate that NAFLD may occur "naturally" in aged zebrafish, while we found no reports indicating this phenomenon in the literature. Combined diet-and-exercise treatment can reverse this phenotype. Given that 14-months-old zebrafish are still reproductively-active and do not exhibit signs of aging, this finding suggests an idiopathic nature of NAFLD.

Identification and Characterization of Nuclear IGF1R

Ravid Solomon Zemler, Haim Werner
Department of Human Molecular Genetics and Biochemistry, Tel Aviv University, Israel

Background: The insulin-like growth factor-1 receptor (IGF1R) is a tyrosine kinase receptor that mediates the mitogenic effects of IGF1 and IGF2. Ligand association leads to IGF1R autophosphorylation and activation of multiple downstream signaling pathways, which are important for cell survival and growth. In addition to the classical roles associated with cell-surface receptors we and other laboratories recently showed a novel nuclear (genomic) role for the IGF1R. Specifically, we identified a SUMO-dependent mechanism of nuclear IGF1R translocation in breast cancer cell lines. In addition, we demonstrated that nuclear IGF1R may act as a transcriptional activator, including autoregulation of its cognate promoter. The aim of the present study was to characterize and analyze the mechanisms associated with translocation of IGF1R into the nucleus in human cells and to investigate the roles of nuclear IGF1R.

Materials and methods: Our working models are IGF1R-expressing non-carcinogenic MCF10A and carcinogenic MCF7 human breast cancer cell lines. To assess the nuclear translocation of the IGF1R in MCF10A cells, we conducted cell fractionation studies followed by Western blots or immune-precipitation (IP) assays. In addition, to directly identify the IGF1R in the nucleus we conducted immunofluorescence assays. To assess the impact of IGF1R levels on nuclear expression, cells were transfected with IGF1R siRNA or non-targeting (NT) siRNA for 48 h or treated with IGF1.

Preliminary Results: As revealed by cell fractionation and immunofluorescence assays, IGF1R was present in the nucleus of both MCF10A and MCF7 cells. In IP assays we observed that indeed IGF1R was expressed in the nuclei of both cells, although IGF1R levels were significantly higher in nuclei of MCF7, compared to MCF10A, cells.

Conclusions: Our preliminary data suggest that nuclear translocation of the IGF1R constitutes a novel and, potentially, important mechanism with physiological relevance in both carcinogenic and non-carcinogenic cells.

Combination of mTOR Inhibitors RAD001 and Torin1: Differential Mechanisms Converge to Superior Potency

Hadara Rubinfeld[1], Ortal Cohen[1], Adi Kammer[1], Moshe Hadani[2], Ilan Shimon[1]

[1]*Institute of Endocrinology, Felsenstein Medical Research Center, Tel Aviv University, Israel*

[2]*Neurosurgery Department, Sheba Medical Center, Tel Aviv University, Israel*

Despite the success in treatment of some cancers, the efficacy of the mTOR inhibitors, rapalogs, as anti-cancer therapeutics has been limited. This may be explained by several observations shown in many models, among them suppressions of feedback loops which cause compensatory overactivation of upstream elements of the PI3K/Akt/mTOR pathway. We undertook to examine the effects of Torin1, a second generation selective ATP-competitive mTOR inhibitor in non-functioning pituitary tumor cells. During characterization of the molecular mechanisms that mediate Torin1 actions, there seemed to be a rationale for combining it with rapalogs. Combined treatments of Torin1 and RAD001 induced a pronounced reduction in cell growth and viability of both MtT/E pituitary cell line and human derived non-functioning pituitary cells, superior to each drug alone. The combined treatment decreased Cyclin D3 and p21/CIP expression more potently than each individual treatment alone, suggesting that the higher efficacy of the combined treatment is due to a more efficient G0/G1 cell cycle arrest. Interestingly, signaling data do not provide an explanation and some even seem to be opposed to the cellular response potency: Akt-Thr308 phosphorylation was robustly elevated in the combined treatment, accompanied by a reduction of PTEN expression. Phosphorylation of p70S6K was abolished in all individual and combined treatments. The Akt-Ser473 phosphorylation pattern by itself does not provide an explanation as to why the combination is more potent than Torin1 treatment alone. Conclusions: our proposed model suggests that combining Torin1 and RAD001 facilitate the efficient blockade of the mTORC2 complex in pituitary tumor cells which may explain the superior potency observed when the drugs were combined.

Selective PKC Isoforms Play a Role in GnRH-Activation of p38MAPK in Pituitary Gonadotropes

Shany Mugami, Zvi Naor
Biochemistry and Molecular Biology, Tel Aviv University, Israel

GnRH is the first key hormone of reproduction in vertebrates. Here we examine the role of PKC isoforms (PKCs) in GnRH-stimulated p38MAPK activation in αT3-1 and LβT2 gonadotrope cells. Incubation of the cells with GnRH resulted in a relatively slow increase in p38MAPK activation in αT3-1 cells with a more rapid response in LβT2 cells. The PKC activator PMA gave a slow response in both cells. Gonadotropes express conventional PKCs (cPKC) α and βII, novel PKCs (nPKC) δ, ε and θ, and atypical PKC (aPKC) ι/λ, while GnRH activates PKCα, PKCβII, PKCδ and PKCε. The use of dominant negative plasmids for the various PKCs and selective peptide inhibitors for the receptors for activated C kinase (RACKs **(**, has revealed that PKCα, PKCδ and PKCε contributed 32, 20 and 22.5% respectively, to GnRH-induced p38MAPK activation in αT3 cells, while PKCβII actually inhibited the response by 20%. PMA recruited PKCα, PKCβII, PKCδ and PKCε by 35, 30, 25 and 22% respectively in the same cells for p38MAPK activation. A larger contribution was observed in GnRH to p38MAPK signaling in LβT2 cells. PKCα, PKCβII, PKCδ and PKCε mediated the response by 58, 50, 52 and 42%, respectively, while the same isoforms contributed 52, 48, 38 and 42% respectively to PMA-induced p38MAPK activation in LβT2 cells. The paradoxical findings that specific PKCs activated by GnRH and PMA play a differential role in p38MAPK activation in a ligand-and cell context-dependent manner, may be explained by differential localization of the PKCs. Finally, we followed the fate of p38MAPK in GnRH-stimulated cells. p38MAPK resides in the cell membrane of resting LβT2 cells, is relocated to the nucleus by GnRH (~5 min) and further relocated to cell ruffles, filopodia and lamellipodia apparently for cell migration.

Endocrine Disruptors Burden in Vegetarians/Vegans is Characterized by a Lower Exposure to Phthalates but not to Bisphenol A: Results of the Amirim Study

Karen Tordjman[1], Laura Grinshpan[1], Lena Novack[2], Naftali Stern[1], Thomas Goen[3], Dar Segev[4], Tamar Berman[5]

[1]*Institute of Endocrinology, Tel Aviv Sourasky Medical Center, Israel*
[2]*Epidemiology, Faculty of Health Sciences Ben Gurion University, Israel*
[3]*Occupational Social and Environmental Medicine, University of Erlangen-Nurenberg, Germany*
[4]*Biotechnology Engineering, Braude College, Israel*
[5]*Environmental Health, Ministry of Health, Israel*

Background and Aim of Study: Unknown a century ago, endocrine disrupting chemicals (EDCs) are now ubiquitous. Mostly entering through the mouth, they are believed to contribute to a number of human diseases such as infertility, obesity, and diabetes. The first Israeli human biomonitoring study (IBMS) recently demonstrated and excess burden for a number of EDCs in the Israeli population compared to that of the US and Canada.

We aimed to test the hypothesis that a vegetariand/vegan diet is associated with a lower burden of exposure to man-made EDCs.

Subjects and Methods: 42 residents of the vegetarian village of Amirim, 29 vegetarians and 13 vegans, answered a lifestyle questionnaire, and underwent a detailed 24 h dietary recall. Concentrations of 11 phthalate metabolites, and that of Bisphenol A (BPA) were determined by tandem mass spectrometry on a morning spot urine. Geometric means of creatinine-adjusted concentrations were compared to those of the Jewish population from the IBMS.

Results: Urinary BPA was no different in the Amirim sample than in the general population. In contrast, 5-OHMHEP and 7-oxoMiNP, metabolites of the high molecular weight (HMW) phthalates DHEP and DiNP (mostly used in PVC manufacturing, toys, and food packaging) were 31-41% lower in Amirim residents (P=0.001 and P0.001, respectively). In men only, the HMW phthalate MBzP (also used in PVC) was 36% lower in Amirim residents (P=0.03). While the low molecular weight phthalates DnBP and DiBP, used in cosmetics, medications and the food industry, were 17-35% lower in Amirim women than in the female IBMS population. HMW phthalate metabolites tended to be lower in vegans than in vegetarians. DHEP metabolites excretion was globally associated with dairy products consumption.

Conclusions: In the absence of much awaited industry regulation, a vegetarian/vegan diet may achieve a moderate reduction in exposure to phthalates, but not to all EDCs.

A Large Cohort of Adults with Central Diabetes Insipidus

Hiba Masri-Iraqi[1], Dania Hirsch[1,2], Dana Herzberg[1], Avner Lifshitz[1], Gloria Tsvetov[1,2], Carlos Benbassat[1,2], Ilan Shimon[1,2]
[1]*Beilinson Campus, Rabin Medical Center, Israel*
[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

Background: Central Diabetes Insipidus (CDI) is a rare heterogeneous condition with a variety of underlying causes. Data on clinical presentation and long-term course is limited especially at adulthood. The aim of the study was to identify the clinical presentation and follow up of a large cohort of adults with CDI.

Methods: Data on demographic characteristics, presentation, imaging, pituitary axes affected, treatment and complications collected from the files of 71 adults followed at a referral endocrine clinic.

Results: This series includes 41 females and 30 males (current mean age, 46±15 years) diagnosed with CDI at the age of 29.2±19.7 years. Twenty eight were identified at childhood. Most patients (39/71) developed CDI following surgery for a sellar mass, the leading tumor in children was craniopharyngioma (11/17 cases) and GH-secreting adenoma (all 7 patients) in adults. Ten patients were diagnosed with Langerhans Histiocytosis(5/10 at childhood). Twelve cases (17%) were idiopathic, half diagnosed at childhood, 9/12 had normal anterior pituitary function. Other etiologies included sarcoidosis, lymphocytic hypophysitis and head trauma. Altogether 73% of the patients had at least one anterior pituitary axis affected; 58% had growth hormone deficiency, 56% hypogonadism, 55% central hypothyroidism, and 44% ACTH-cortisol deficiency; 31% had panhypopituitarism. All patients but one were treated with vasopressin preparations, nasal spray as the leading one. Hyponatremia developed in 32 patients, most were managed without hospitalization, five had severe hyponatremia (150 mEq/l) was recorded in 5 patients. Altogether, calculated complication rate was 10/1250 treatment years.

Conclusions: Adult CDI is a variable disease with different etiologies. Most patients have anterior pituitary dysfunction. Patients are usually stable on long-term treatment with relatively low complication rate.

The Roles of the GH/IGF-1 Axis in Bone Metabolism during Development and Aging

Shoshana Yakar
Department of Basic Science and Craniofacial Biology New York University College of Dentistry New York, David B. Kriser Dental Center, USA

Growth hormone (GH), as the name suggets is a major regulator of body size, body composition, and bone growth. GH is the prime regulator of insulin-like growth factor-1 (IGF-1) in the liver, which acts on tissues in endocrine and autocrine/paracrine fashion. Studies in human and animal models have shown that reductions in serum IGF-1 levels lead to impaired skeletal morphology and significant reductions in bone mineral density (BMD) with mild reductions in linear growth. In contrast, elevations in serum IGF-1 enhance skeletal properties and BMD. Animal models where tissue igf-1 gene expression was ablated show that bone GH/IGF-1 axis is crucial for neonatal and early postnatal bone growth as well as for maintaining trabecular bone volume during advanced aging. However, elevated serum IGF-1 can fully compensate for a postnatal absence of tissue IGF-1 when the GH receptor (GHR) pathway is intact. In contrast, serum IGF-1 cannot compensate for the absence of tissue GHR action. Receptors for both IGF-1 (IGF-1R) and GH are found on all bone cells (osteoblast, osteoclast, and osteocytes). Studies in mice with osteoblast-specific IGF-1R ablation revealed a reduced bone formation rate and mineralization defects during growth (3–6 weeks of age). In contrast, the overexpression of IGF-1 in osteoblasts increased the bone formation rate by two-fold, leading to a 30% increase in trabecular bone volume. Our recent studies with osteocyte-specific-GHR ablated mice revealed that osteocyte-GHR regulates radial bone growth. We found that osteocyte GHR plays significant roles in integration of GH and parathyroid hormone (PTH)- signals to regulate bone accrual during growth.

The Role of the Anti-aging Gene Sirtuin1 in Osteoporosis: From Mice to Women

Rivka Dresner-Pollak
The Hadassah University Hospital-Ein Kerem, Department of Endocrinology

Bone loss is an inevitable consequence of aging. While diabetes and dementia occur in some older individuals, osteoporosis occurs virtually in everyone if they live long enough. Thus, key regulators of aging are likely to play a role in osteoporosis pathophysiology. The sirtuin family of NAD+-dependent deacetylases was found to play a key role in aging and in age-associated diseases. There are seven mammalian sirtuins which differ in their cellular location and function. Sirtuin1 (Sirt1), the most investigated sirtuin, was shown to regulate inflammatory pathways, glucose and lipid metabolism, apoptosis and DNA damage repair.

Others and we have previously shown that Sirt1 regulates bone mass. Global Sirt1-over expression confers protection against age-associated bone loss in old male mice, while Sirt1 haplo-insufficiency (global deficiency is lethal) results in low bone mass and bone formation. Targeted deletion of sirt1 in osteoclasts, osteoblasts and mesenchymal stem cells leads to low bone mass and impaired bone structure. We have discovered that Sirt1 is a negative regulator of sost gene expression by deacetylating histone 3 at its promoter. Sost encodes for sclerostin, an inhibitor of bone formation, and antibodies against sclerostin is an emerging novel bone anabolic therapy for osteoporosis currently under investigation in clinical trials. Moreover, we have shown that pharmacologic activation of Sirt1 down-regulates sclerostin and restores bone mass and strength in ovariectomized mice, the classic animal model of osteoporosis.

To investigate the relevance of these findings to humans, we studied Sirt1 and sclerostin expression at the femoral neck in female patients who underwent hip operations for a sub-capital osteoporotic hip fracture and hip replacement for osteoarhtritis. Reduced Sirt1 and increased sclerostin expression were found in osteoporotic compared to osteoarthritic patients. Moreover, the administration of a Sirt1 activator to human primary bone marrow mesenchymal stem cells obtained from these patients decreased sclerostin expression.

The anti-aging gene Sirt1 probably plays a role in osteoporosis and its activation is plausible novel pathway to generate novel bone anabolic therapies for osteoporosis.

Nutritional Aspects of Skeletal Development

Efrat Monsonego-Ornan
Biochemistry and Nutrition, The Hebrew University, Israel

While the connection between under-nutrition and growth retardation is well documented, the opposite connection between over-nutrition and bone development was barely studied. For instance, obese children grow faster in height than normal-weighed children, and prospective studies demonstrated an over-presentation of obese children amongst fracture cases. Furthermore, little is known about the direct effect and the underlying cellular and molecular mechanisms of the diet or single nutrients on the cells of the developed bone.

We analyzed in depth the effect of childhood obesity on young bone elongation and bone quality. Multiple complementary in-vivo models were utilized to characterize in details the growth-plate phenotype as well as the bone structure and mechanical properties. The various models we used are: pharmaceutical inhibition of leptin signaling (by leptin antagonists) and various types of obesogenic diets such as high fat diet (HFD). We found that obesity in young age affected both bone elongation and bone quality. Furthermore, the type of the diet, distinctly from its obesogenic effect, modified bone development and quality. For instance, while HFD based on poly unsaturated fatty acids impairs bone morphology; omega-3 fatty acids improves it. Our studies demonstrated the involvement of metabolic signals such as adiponectin, leptin and IL1□. We discovered a novel mechanism by which osteocalcin shifts chondrocytes toward glycolytic breakdown of glucose and stimulates their calcification, in a HIF-1α-dependent manner. Based on these findings, we suggest that the metabolic status in obesity and the specific component in the diet affect directly the metabolic state of bone cells, leading to accelerated bone elongation and modified processes of bone formation and resorption. This topic is of tremendous importance for both basic and applicative scientists in the fields of pediatrics, nutrition, endocrinology, bone health and development.

Symposium 2: Genetic Testing in Endocrine Tumors: Indications and Clinical Implications Regarding Prognosis and Treatment

Pituitary Tumors

Ilan Shimon
Department of Endocrinology, Diabetes & Metabolism, Rabin Medical Center-Beilinson Campus

Pituitary adenomas are benign intracranial neoplasms. The vast majority of these tumors occurs sporadically, and is not part of established syndromes, nor associated with known genetic mutations. Somatic mutations in the gsp oncogene (G-protein a-subunit) are responsible for 40% of GH-secreting adenomas. Similar post-zygotic genetic aberration is identified in patients with McCune-Albright syndrome that may develop GH or GH/PRL tumors. Germline mutations in MEN1 (encoding menin) and in CDNK1B (encoding p27 cell cycle inhibitor) result in multiple endocrine neoplasia type 1 and type 4, respectively. These syndromes are characterized by the occurrence of anterior pituitary adenomas among other endocrine tumors, but are responsible for less than 3% of patients harboring pituitary tumors. Germline mutations in AIP (aryl-hydrocarbon receptor-interacting protein) may appear in a subset of familial isolated pituitary adenoma (FIPA) families with GH- or PRL-adenomas. They are usually detected in young patients with large invasive tumors and family history of similar tumors, but may also appear in ~4% of patients with sporadic adenomas, and 8-20% in young adults or children with macroadenomas. Recently, microduplication on chromosome Xq26.3 was observed in young patients with acromegaly and gigantism (X-linked acrogigantism), both as sporadic or familial cases. Conclusions: most pituitary adenomas are sporadic without known genetic mutations. Several germline mutations associated with endocrine syndromes were identified in the last 10-15 years. These are responsible for familial clusters of pituitary adenomas, usually GH- or PRL-tumors detected more in children and young adults.

Genetic Testing in Differentiated Thyroid Carcinoma: Indications and Clinical Implications Regarding Prognosis and Treatment

Sagit Zolotov

Institute of Endocrinology, Diabetes & Metabolism, Rambam Medical Center

Differentiated thyroid cancer (DTC) is a common and diverse endocrine malignancy. DTC is mostly diagnosed by cytological evaluation. However, in up to 30% of the cases the cytological result is either unsatisfactory or non-diagnostic. In addition, DTC can range from a curable disease to an aggressive and therapy-resistant cancer. Genetic testing of tumor cells can assist clinicians in the diagnosis and characterization of DTC in the individual patient. Genetic changes leading to DTC include mutations, gene copy-number gain and aberrant gene methylation that ultimately lead to profound dysregulation in major signalling pathways promoting tumorogensis. These molecular changes may serve as key diagnostic and prognostic biomarkers and as a valuable and informative platform for personalized medical care of DTC patients.

Autophagy Is A Major Regulator Of β-Cell Insulin Homeostasis

Gil Leibowitz

[1]*Department of Endocrinology, Hadassah Hebrew University Medical Center*

The pancreatic β-cell synthesizes and secretes large amounts of insulin to maintain euglycemia under varying metabolic conditions. Newly synthesized proinsulin on its way from the ER to secretory granules is subjected to oxidative folding, intracellular trafficking and packaging in granules for further processing and secretion. Little is known on the role of protein degradation pathways in the regulation of insulin production and secretion. Using biochemical, imaging and genetic approaches, we show that proinsulin is robustly regulated by lysosomal degradation. Short-term inhibition of lysosome acid hydrolases in islets and β-cells and knockdown of autophagic genes required for autophagosome maturation markedly increased proinsulin content and secretion. Proinsulin mutants that are irreparably misfolded and trapped in the ER were not degraded by lysosomes, suggesting that proinsulin transport to autophagosomes and/or lysosomes occurs downstream to the ER, most probably at the trans-Golgi network, as suggested by colocalization studies. In addition, we show that autophagy regulates cytoskeleton remodeling, thereby modulating proinsulin degradation and insulin secretion. F-actin depolymerization inhibited autophagy, resulting in increased proinsulin level with appearance of proinsulin-containing P62/SQSTM1+ aggregates along with increased insulin secretion. Conversely, inhibition of autophagy in β-cells induced F-actin depolymerization in vivo and amplified insulin secretion. We conclude that crosstalk between actin network and autophagy constitutes a central node in the regulation of β-cell proinsulin degradation and insulin secretion.

Reading DNA (Whole Exome Sequencing) and RNA (Expression and Regulation) for Personalized Medicine

Noam Shomron
Faculty of Medicine, Tel Aviv University, Israel

Next-generation sequencing (NGS; also known as Deep Sequencing) has probably been the most important tool for genomic research over the past few years. NGS has led to numerous discoveries and scientific breakthroughs in the genetic field. The sequencing technology is shifting from the research laboratory to the clinical diagnostic arena. Multiple NGS protocols are used for reading DNA and RNA. These allow a comprehensive view of the cell, tissue or organ under investigation with actionable relevance for the patient leading to efficient Personalized Medicine.

Application of Proteomics Technology to Cancer Research, from Diagnostics to Personalized Therapy

Tamar Geiger
Human Genetics, Faculty of Medicine, Tel Aviv University, Tel- Aviv, Israel
Sackler School of Medicine

Mass-spectrometry (MS)–based proteomics, in analogy to the term 'genomics', aims at the identification of the assortment of proteins expressed in a biological system, their interactions and modifications. The proteins are the main active entities in the cells; therefore they better reflect the cellular phenotype than mRNA. Previously, proteomics was limited to simple samples of only few proteins, however in recent years, developments in the MS technology, sample preparation techniques and computational analysis opened new possibilities for quantitative genome-scale proteomics. These developments increased the proteome coverage, to routinely identify thousands of proteins in single experiments; increased the throughput to allow comparison of large sample cohorts, and provided accurate quantification of the proteins. Our research combines basic and translational research, with technology development to open new possibilities to apply proteomics to tissues and body fluids. We apply these techniques to follow breast cancer progression and identify cancer biomarkers and novel drug targets. We identified a proteomic signature that discriminates between breast cancer stages, and can determine, based on the primary tumor, whether it has metastasized or not. This work shows the potential of clinical proteomics to unravel unique and robust breast cancer signatures, with the potential to alter breast cancer diagnosis and treatment.

Disorders of Sex Development

Kenneth McElreavey
France, Human Developmental Genetics Institut Pasteur, France

Next generation sequencing technologies are dramatically changing biomedical research and patient diagnosis. The reducing costs of sequencing as well as robust experimental and computational protocols means that this technology is becoming available to most laboratories. However, the identification of disease-causing mutations in individuals with pediatric endocrine diseases is challenging. Next generation sequencing has revealed an unanticipated degree of genetic variation with many predicted loss-of-function mutations present in the general population that can hinder the phenotypic interpretation. Exome sequencing has emerged as a very powerful tool to identify the genetic basis of rare human Mendelian disorders. The current focus on exomes, rather than the entire genome should be sufficient to uncover a great number of relevant targets in a cost-efficient and easily interpretable manner. This approach is particularly attractive for mutation detection in cases of Disorder of Sex Development (DSD) since these conditions are very difficult to study using classical genetic approaches. Excluding cases where the biochemical profile indicates a specific error in steroidogenesis, it is estimated that a specific molecular diagnosis is obtained in 20% of DSD cases and that only 50% of 46,XY children with DSD will receive a definitive clinical diagnosis. We have now performed exome sequencing in >120 cases of DSD using the Illumina HiSeq2000 platform. In this talk I will summarise our main findings and highlight some of the challenges and surprises that have resulted from this approach.

A child with 46XY DSD – should sex reassignment be considered?

Liat de Vries
The Jesse Z and Sara Lea Shafer Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel

A 3-year old girl presented with clitoromegaly, noticed by the mother. She is a third child, born following an uneventful pregnancy to healthy, first-cousin parents of Christian Arab origin. She has two older healthy sisters with normal genitalia.

Physical examination was unremarkable except for palpable gonads in the inguinal region. Pelvic ultrasound and MRI did not demonstrate Mullerian structures and gonads were visualized in the inguinal canal. Karyotype was 46, XY. The stepwise work-up leading to diagnosis will be described. The dilemma regarding sex-reassignment in a 3-year old girl will be discussed. Considerations in decision-making on sex-reassignment are based on: (1) specific aspects of the condition itself; (2) considerations related to corrective surgery, fertility and future sexual satisfaction in the child; and (3) cultural and social considerations.

Uterine Removal in Mixed Gonadal Dysgenesis / XY Partial Gonadal Dysgenesis, Raised as Males: Should we Leave it for Them to Decide?

Anat Segev-Becker, Sarah Meisler,
Dana Children's Hospital

Background: Mixed gonadal dysgenesis (MGD) is the second most frequent cause of XY disorders of sex development (DSD). Genotype is either X/XY or XY, while the phenotype ranges from partial to complete gonadal dysgenesis, and from female to male external genitalia. Müllerian remnants are present in these patients because of insufficient or untimely fetal secretion of Müllerian inhibiting factor (MIF).

Aim: To assess the therapeutic policy of physicians of patients with MGD regarding the removal of the Müllerian remnants.

Methods: Physicians who entered data on gonadal dysgenesis into the I-DSD registry were e-mailed and asked, "When, if ever, do you recommend the removal of the rudimentary uterus from boys with gonadal dysgenesis associated with a X/XY or XY karyotype?"

Results: Thus far, 20 physicians have responded. Thirteen promote removal of the Müllerian remnants in childhood at the same time as the removal of the dysgenic gonads because of the risk of recurrent infections, uterine adenocarcinoma, or in order to prevent any ambiguity in gender identity. Two physicians advise delaying removal until adulthood for patient consent and understanding, four physicians do not recommend removal unless there is a medical reason, and two physicians assess their patients case by case.

Conclusion: Even though there is a shift from the earlier “optimal gender policy” (the paternalistic approach) to a “full consent policy”, most of the responding physicians contend that the Müllerian remnants should be removed as soon as the decision is made to raise the child as male.

Clinical Approach to Primary Hyperaldosteronism (from Screening Tests to AVS)

Silvia Monticone
Italy, University of Torino

Introduction: Primary aldosteronism (PA) is the most common form of endocrine hypertension that is associated to an increase rate of cardiovascular events. PA detection provides an opportunity for a targeted treatment (surgical for aldosterone producing adenoma and medical with mineralocorticoid receptor antagonists for bilateral adrenal hyperplasia). According to the Endocrine Society Guidelines PA diagnosis is made following a rigorous three step flow-chart comprising screening, confirmation/exclusion testing and subtype differentiation.
Key learning points.
The categories of hypertensive patients that should be screened include resistant hypertensives, hypertensives grade 2/3, hypertensive with hypokalaemia or adrenal incidentaloma and hypertensives with risk of familial hyperaldosteronism. Aldosterone/renin ratio is the most reliable means for PA screening and should be performed under strictly controlled conditions. After a positive screening test, the patient should undergo a confirmatory test (fludrocortisone suppression test, intravenous or oral saline load test, captopril challenge).
After PA confirmation, subtype differentiation should be performed to allocate the patient to the correct management. All patients affected by PA should undergo adrenal CT scannig, as the initial study in subtype differentiation. After CT scan, it is recommend that all patients for whom the surgical treatment is practicable and desired should undergo adrenal vein sampling (AVS) as the gold standard to differentiate unilateral from bilateral disease. AVS should be performed by an expert radiologist using a predefined protocol for the procedure and results interpretation.
Controversies in the field.
Consensus is missing on the following points: best cut-off for screening and inclusion of absolute aldosterone values; cut-offs for confirmatory tests; conditions of the AVS (cosynthropin or unstimulated procedure) and interpretation (best selectivity and lateralisation index).

Growth Factors, Hormones and Cancer

Mixed Growth Hormone and Thyrotropin Secreting Pituitary Adenoma Treated by Octreatide, Cabergoline and Pegvisomant Combination

Michal Gershinsky[1,2], Bardicef Orit[1], Sophia Ish-Shalom[1,3]
[1]*Endocrinology, Linn Medical Center, Clalit Health Services, Israel*
[2]*Faculty of Medicine, Technion, Israel*
[3]*Endocrine Research Center, Linn Medical Center, Clalit Health Services, Israel*

Acromegaly is usually caused by a GH-secreting pituitary macroadenoma. GH-producing adenomas often co-express prolactin (PRL) and, much less frequently, thyroid stimulating hormone (TSH). These tumors often present a therapeutic challenge in attaining an appropriate control of plurihormonal hypersecretion and tumor mass reduction.

We describe a 50-years old man, who presented in 1997 with extreme fatigue and headache. Clinical examination revealed tachycardia, hypotension, acromegalic features and diffuse goiter. Hormonal assessment was consistent with hypoadrenalism, hypogonadism, normoprolactinemia, acromegaly and secondary hyperthyroidism. Visual field examination revealed right temporal defect. Pituitary macroadenoma (28X30X41 mm) with suprasellar extension, optic chiasm elevation and sphenoid sinus invasion was demonstrated on MRI-scan.

Only partial tumor resection was possible by transsphenoidal approach due to severe tumor fibrosis. The patient refused transcranial surgery. Radiation therapy was not possible due to optic chiasm proximity.

Octreotide and methimazole treatment had not achieved thyrotoxicosis and acromegaly control. Cabergoline addition induced euthyroidism and allowed methimazole cessation. Octreatide and cabergoline combination caused significant tumor shrinkage (28X28X16 mm), but severe GH hypersecretion required GH-receptor blocker (pegvisomant) addition. IGF-1 levels decreased from 92 to 38 nmol/l (N 6-28 nmol/l), yet adequate acromegaly control had not been achieved, and in 2014 fractionized stereotactic radiotherapy was performed.

This case illustrates several issues in the treatment of GH/TSH producing macroadenoma with possible future implications:

- Octreotide and cabergoline combination treatment was effective for significant size reduction of a fibrotic, surgically unresectable macroadenoma, which allowed subsequent tumor irradiation
- It induced euthyroidism in a patient with mixed GH /TSH, non-prolactin secreting tumor.
- This is the first report of triple pharmacotherapy with octreatide, cabergoline and pegvisomant in normoprolactinemic patient with mixed GH and TSH secreting tumor. Pegvisomant addition induced significant IGF-1 reduction, while awaiting radiation therapy effects.

Comparative Effects of Highly Potent New 1,25-Dihydroxyvitamin D Analogs on Differentiation of Human Acute Myeloid Leukemia (AML) Cells

Matan Nachliely[1], Andrew Kutner[2], Michael Danilenko[1]
[1]*Department of Clinical Biochemistry & Pharmacology, Ben Gurion University of the Negev, Israel*
[2]*Chemistry, Pharmaceutical Research Institute, Poland*

1,25-dihydroxyvitamin D (1,25D) is known as a powerful differentiation inducer in various types of cancer cells, including AML cells. This effect can force AML cells to bypass the differentiation barrier that characterizes this disease. However, 1,25D concentrations required to induce terminal differentiation of AML cells can cause lethal hypercalcemia *in vivo*. Here we determined the differentiation-inducing effects of new low-calcemic double-point modified 1,25D analogs, PRI-5201 and PRI-5202 [Pietraszek et al. (2013) Steroids 78:1003-1014], on HL60 and U937 human AML cells, in comparison to their direct precursors (PRI-1906 and PRI-1907, respectively) and the parent compound (1,25D). We also compared the ability of all the vitamin D derivatives (VDDs) tested to cooperate with rosemary polyphenol carnosic acid (CA), which has been shown in our studies to potentiate the antileukemic effects of VDDs. The results demonstrated the following order of VDD potency: 5202 1907 5201 1906 1,25D, as measured by flow cytometric determination of the expression of cell surface markers of myeloid differentiation. Particularly, the sensitivity of AML cells to PRI-5201 and PRI-5202 was 1-2 orders of magnitude higher compared to that of 1,25D. Furthermore, all the synthetic 1,25D analogs tested retained the capability of cooperating with CA, which markedly enhanced the differentiating effect of sub-nM concentrations of the VDDs in a synergistic manner. These data suggest that the new synthetic 1,25D analogs are promising candidates for further mechanistic and translational studies in AML (Supported by the Israel Science Foundation grant 635/11).

BMI in 2.3 Million 16-19 Year Old Adolescents and Cardiovascular Mortality in Young and Middle-Aged Adults

Gilad Twig[1,2,3,4], Hagai Levine[5], Gal Yaniv[2,3], Adi Leiba[1,2], Estela Derazne[2], Dorit Tzur[2], Nehama Goldberger[6], Dana Ben-Ami Shor[1], Arnon Afek[6], Ari Shamis[1], Ziona Haklai[6], Jeremy Kark[5]

[1]*Department of Medicine, Sheba Medical Center*

[2]*The Surgeon General Headquaters, The Israel Defense Forces Medical Corps*

[3]*Talpiot Medical Leadership Program, Sheba Medical Center*

[4]*Department of Endocrinology and Metabolism, Sheba Medical Center*

[5]*Hadassah School of Public Health, Hebrew University*

[6] *Israel Ministry of Health*

Our objective was to assess the association of BMI in 16-19 year-old Israeli adolescents with subsequent cardiovascular mortality. BMI of 2,298,130 adolescents (60% males, age 17.4±0.3 years), measured prior to military service between 1967 and 2010, was grouped according to the US CDC percentiles and linked with national mortality records. The primary outcomes of the study were mortality due to coronary heart disease (CHD), sudden death (SD), and cerebrovascular events (CVA). Cox proportional hazards models were applied.

During 45,729,521 person-years of follow-up (mean 19.9±12.0 y, maximum 44 y) there were 1497, 528 and 893 deaths from CHD, CVA and SD, respectively. There was a graded increase in CHD mortality from the 5^{th}-25^{th} BMI percentiles onwards. Obesity (95^{th} percentile) was associated with hazard ratios of 5.1 (95%CI=4.0-6.4, p0.001), 2.5 (95%CI=1.6-3.9, p0.001) and 2.1 (95%CI=1.5-3.0, p0.001) for CHD, CVA and sudden deaths, respectively, after adjustment for sex, age, birth year, education, socioeconomic status, country of origin and height. For the latter model, overweight (85^{th}-95^{th} BMI percentiles) was associated with HR of 3.1 (95%CI=2.5-3.7, p0.001), 1.7 (95%CI=1.2-2.4, p=0.002) and 1.4 (95%CI=1.1-1.8, p=0.02) for CHD, CVA and sudden deaths, respectively. Findings persisted when the analysis was restricted to those with unimpaired health status (n=1,669,687, including 50,002 obese) and when the follow-up ceased at 45 years of age.

When BMI of 17.5-19.9 kg/m^2 was used as a reference, BMI of 20-22.4 kg/m^2 was already associated with increased adjusted CHD mortality (HR 1.2, 95%CI= 1.1-1.4, p=0.006), and BMI of 22.5-24.9 kg/m^2 with higher CVA and SD mortality (HR=1.3, 95%CI=1.1-1.7, p=0.02 and HR=1.4, 95%CI=1.1-1.6, p=0.002, respectively). Therefore, BMI in adolescence, well-within the currently accepted normal range, predicts cardiovascular mortality. The population attributed risk for CHD mortality that is related to obesity with the current prevalence of adolescence obesity in Israel is estimated by 26.0% (95%CI=20.8%-31.6%).

A Novel Function of Steroidogenic Acute Regulatory (StAR) Protein as an Anti-Apoptotic Survival Factor in the Failing Heart

Eli Anuka[1], Naomi Melamed-Book[2], Nili Naftali-Shani[3,4], Radka Holbova[3,4], Jonathan Leor[3,4], Joseph Orly[1]

[1]*Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*

[2]*Bio-Imaging Unit, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*

[3]*Tamman Cardiovascular Research Institute and Sheba Center of Regenerative Medicine, Stem Cells and Tissue Engineering, Sheba Medical Center, Israel*

[4]*Neufeld Cardiac Research Institute, Sackler Faculty of Medicine, Tel Aviv University, Israel*

StAR is a mitochondrial protein essential for supply of cholesterol for steroid synthesis in mitochondria of specialized cells in the adrenal cortex and the gonads. Surprisingly, we have recently reported that StAR is also expressed in non-steroidogenic cells in the left ventricle (LV) of mouse heart following experimental myocardial infarction (MI). Detailed spatio-temporal analyses of StAR expression show a tansient peak of the protein expression in non-myocytes 'fibroblast-like' cells limited to the infarct area. Co-expression of StAR with cellular markers suggest that the origin of StAR expressing cells can be resident cardiac fibroblasts, endothelial cells undergoing mesenchymal transition and blood born bone-marrow derived cells (monocytes/macrophages) all recruited to the infarct zone at the inflammatory phase of the infarct healing. Studies of cardiac fibroblasts put in culture suggest that StAR endows the cells with anti-apoptotic robustness, a quality that probably allows them to survive the detrimental infarct environment and launch the life-saving wound-healing process. Our in situ studies suggest that the mechanism of StAR function includes the inhibition of pro-apoptotic BAX translocation to the mitochondrial outer membrane where it sets in motion the apoptotic cascade. In heterologous cell model, loss-of-function human StAR mutants failed to reverse the apoptotic fate of HeLa cells treated with a strong apoptogen, staurosporine. To examine if cholesterol homeostasis plays a role in the cell responses to apoptotic stress, we applied live confocal imaging studies and detergent resistant membrane (DRM) analyses to show that StAR (but not its mutants) triggers the enrichment of plasma membrane with cholesterol-rich lipid rafts (caveolae) known to transfer cholesterol to intracellular compartments. We therefore hypothesize that, similar to apoptosis-resistant hepatoma cells shown to increase their cellular cholesterol content, it is StAR that protects the wound healing myofibroblasts of a failing heart by mobilizing cellular pools of cholesterol to the mitochondria.

Expression of Anti-Apoptotic Steroidogenic Acute Regulatory Protein (StAR) in Non-Steroidogenic Cardiac Myofibroblasts

Talya Shen[1], Tiki Sasson[1], Eli Anuka[1], Naomi Melamed-Book[2], Yael Friedmann[2], Joseph Orly[1]

[1]*Department of Biological Chemistry, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*

[2]*The Bio-Imaging Unit, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel*

StAR is essential for steroid hormones synthesis in mitochondria of specialized adrenal cortex and gonadal cells. Unexpectedly, we have recently reported that StAR is also expressed in non-myocyte mouse cardiac cells exclusively observed at the site of tissue injury following experimental *m*yocardial nfarction (MI). Intriguingly, in the absence of *de novo* cardiac- steroidogenesis, StAR was expected to fit a non-steroidogenic new role. In order to explore the nature of the StAR expressing cells, we set to grow primary 'fibroblasts' isolated from adult rat heart, and characterize their fate throughout11 days growth with TGF-b1; this cytokine is pivotal for differentiation of myofibroblasts normally homing to the infarcted tissue of the left ventricle (LV). Use of confocal microscopy, Western blot analyses and electron microscopy approaches showed that regardless TGF-b1, the cultured cells underwent time-dependent terminal differentiation to become myofibroblasts characterized by expression of typical a-smooth muscle actin, collagen type I, ED-A fibronectin and expression of the focal adhesion protein, talin. Both before and after differentiation, StAR was rapidly induced by treatment with a strong apoptogen, staurosporine, thus recapitulating in culture the appearance of the protein during the MI trauma causing apoptosis and necrosis. Flow cytometry analyses were consistent with the notion that cells expressing StAR were markedly more resistant to apoptosis, suggesting that the novel function of cardiac StAR is to protect the pre-myofibroblasts against cell death when ischemia/hypoxia/ROS environment develops at the site of the MI. Survival of the myofibroblast precursor cells is instrumental for allowing the onset of the post-MI wound healing process. Our studies also hypothesize that a local release of TGF-b1 *in vivo* elicits a proper form of myofibroblast differentiation including the cell compliance to die at the end of the myocardium repair in order to avoid onset of detrimental fibrosis that remains a threat in MI patients.

Regulation of IGF1R Gene Expression and Action by the TMPRSS2-ERG Fusion Protein in Prostate Cancer

Shilhav Meisel Sharon, Haim Werner
Department of Human Molecular Genetics and Biochemistry, Tel Aviv University, Israel

Background: Prostate cancer is one of the leading causes of cancer related death in men. The IGF1R axis plays a key role in prostate cancer. Recurrent gene fusions of the 5` untranslated region of the TMPRSS2 gene to members of the ETS transcription factor family have been identified as key factors in prostate cancer. As a result of the androgen sensitive TMPRSS2 promoter, cells expressing the fusion protein exhibit an androgen-regulated expression of ERG. Consequently, ERG was identified as the most commonly overexpressed oncogene in prostate cancer. The aim of this study was to analyze the involvement of the TMPRSS2-ERG chimera in the regulation of IGF1R gene expression and to investigate the regulation of the IGF1 signaling pathway by TMPRSS2-ERG.

Materials and methods: M12, a prostate cancer cell line, was infected with a TMPRSS2-ERG (T-ERG) expression vector. VCaP, a T-ERG-expressing cell line, was transfected with an siRNA directed against T-ERG. Protein levels were measured by Western blotting. Co-transfections were performed using the T-ERG expression vector along with an IGF1R promoter luciferase reporter plasmid and luciferase activity was measured. Physical interaction between T-ERG and upstream regulatory factor Sp1 was examined by co-immunoprecipitation assays in T-ERG overexpressing cells.

Results: M12 transfectants showed elevated levels of IGF1R protein as compared to control. In VCaP cells, a decreased level of T-ERG was associated with reduced IGF-IR level. In addition, IGF1R promoter activity in T-ERG-expressing M12 cells was higher than in control. Immunoprecipitation assays demonstrated a physicial interaction between T-ERG and Sp1 transcription factor.

Conclusions: Our data indicate that T-ERG positively regulates IGF1R gene expression at the transcriptional level. Results provide support to the notion that the IGF1R gene constitutes a novel downstream target for TMPRSS2-ERG action. Identification of the mechanisms of action of TMPRSS2-ERG in prostate cancer may have important basic and clinical relevance.

Cell Surface Expression of CD24 may Predict Cancer Cells Response to both Insulin and IGF1

Ran Rostoker[1], Sagi Abelson[2], Sarit Ben-Shmuel[1], Inna Genkin[1], Eyal J-Scheinman[1], Keren Bitton-Worms[1], Zila Shen-Orr[1], Avishay Caspi[1], Maty Tzukerman[2], Derek LeRoith[1]

[1]*Diabetes and Metabolism Clinical Research Center of Excellence, Rambam Medical Center and Rappaport Faculty of Medicine and Research Institute, Israel*

[2]*Laboratory of Molecular Medicine, Rambam Medical Center and Rappaport Faculty of Medicine and Research Institute, Israel*

Insulin resistance along with preexisting hyperinsulinemia is believed to be a dominant factor for exacerbating malignancies in diabetic patients through activation of the IR/IGF1R/PI3K/Akt axis. Since the recent failures in clinical trials of therapeutic strategies targeting this axis, the need for predictive biomarkers is essential in order to identify a subset of patients who can benefit from this strategy.

In this study we sorted Mvt1 cells into $CD24^-$ and $CD24^+$ cells. CD24 is considered to be a mammary epithelium stem/progenitor cell marker. Both subsets were morphologically and phenotypically characterized, and tumorigenic capacity was assessed via orthotopic inoculation of each subset into the mammary fat pad of wild-type and MKR mice. The metastatic capacity of each subset was determined with the tail vein metastasis assay. The role of CD24 in tumorigenesis was further examined with shRNA technology. GFP-labeled cells were monitored in-vivo for differentiation. The genetic profile of each subset was analyzed using RNA sequencing.

Here, we demonstrate that $CD24^+$ cells displayed a more spindle-like cytoplasm with high mammospheres efficiency rate. Whereas $CD24^-$ cells were found to be highly insulin and IGF1 sensitive, the $CD24^+$ subset only mildly responsive to both ligands, as indicated by pAkt levels. In-vivo, $CD24^+$ tumors displayed rapid growth in both WT and MKR mice, and were more metastatic than $CD24^-$ cells. Interestingly, $CD24^-$KD in $CD24^+$ cells had no effect both in-vitro and in-vivo on the various parameters studied. RNA-seq analysis revealed enrichment of genes and pathways of the extracellular matrix in the $CD24^+$ cells.

Taken all together, these results indicate that CD24 surface expression identify highly tumorigenic cancer cells, however it is the lack of CD24 expression that may predict favorable outcomes following therapeutic targeting of the IR/IGF1R/PI3K/Akt axis.

Architecture of GnRH-Gonadotrope-Vasculature Suggests a Neurovascular Mode of Gonadotrope Regulation in Zebrafish

Matan Golan[1], Einat Zelinger[2], Yonathan Zohar[3], Berta Levavi-Sivan[1]
[1]*Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*
[2]*Interdepartmental Equipment Unit, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel*
[3]*Department of Marine Biotechnology, University of Maryland Baltimore County and Institute of Marine and Environmental Technology, USA*

Vertebrate reproduction is controlled by gonadotropin-releasing hormone (GnRH) which stimulates expression and secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the adenohypophysis. The function and components of the hypothalamic–pituitary axis are conserved among vertebrates, but in fish a neuroglandular mode of delivery was considered dominant, whereas in tetrapods hypothalamic signals are relayed via the hypophysial portal blood system. By using a transgenic zebrafish model we studied the functional and anatomical aspects of gonadotrope regulation thus revisiting the existing model. FSH cells were found to be situated close to the vasculature whereas the compact organization of LH cells prevented direct contact of all cells with the circulation. GnRH fibers formed multiple synaptic boutons upon reaching the pituitary, but most of these structures were located in the neurohypophysis rather than adjacent to gonadotropes. A close association was observed between FSH cells and GnRH boutons, but only a fifth of the LH cells were in direct contact with GnRH axons. GnRH fibers closely followed the vasculature in the neurohypophysis and formed numerous boutons along these tracts. These vessels were found to be permeable to relatively large molecules, suggesting the uptake of GnRH peptides. Our findings have important implications regarding the differential regulation of LH and FSH and contradict the traditional view of the teleost pituitary that assumes direct innervation of endocrine cells by hypothalamic fibers. Instead, we provide evidence favoring a non-direct, neurovascular control of gonadotropes which is more reminiscent of tetrapods.

The Activity of Recombinant Gonadotropins and their Receptors in the Common Carp

Lian Hollander-cohen, Joseph Aizen, Michal Shpilman, Berta Levavi-Sivan
Department of Animal Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Israel

The Common Carp (*Cyprinus carpio*) is the largest group of cultured aquatic organisms. In Carp aquaculture, In-Vitro Fertilization (IVF) is often used to induce synchronized ovulation. The gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and their receptors play critical roles in vertebrate reproduction. In order to improve the efficiency and productivity of spawning agents in carp we characterize, in-vitro and in-vivo, carp recombinant gonadotropins (recGtH) produced in the methylotrophic yeast *Pichia pastoris* system. Their biological activities were primarily tested on fragments of carp's ovaries in two different developmental stages. In both cases, the recGtHs elicited estradiol secretion. cDNA sequences encoding two carp gonadotropin receptors (FSHR and LHR) have been isolated. An annual profile of mRNA expression in gonad and pituitary tissues showed a rise in the expression of LH/LHR in the mature follicle stage, while surprisingly FSH/FSHR expression also increased in advanced stages of the mature follicle. This might indicate that FSH/FSHR has a specific role – although yet unknown - during the maturation and ovulation and not only during vitellogenesis as shown for other fish species. Receptor transactivation assay of the carp's GtH receptors was studied using transient transfection assays in COS-7 cells. Each of the Carp's recombinant GtHs activated its own cognate receptor; however, we also found promiscuous activation of the FSH receptor by LH. Our findings together with other studies on different fish species highlight the evolutionary origin of the specificity of fish gonadotropin receptors. Recombinant carp LH, when injected to mature female fish significantly enhanced estradiol and DHP secretion, in a similar manner to the hormonal profile of carp pituitary extract. Moreover, some of the treated carps spawned fertile eggs suggesting that this recombinant protein may be a suitable substitute to the spawning agents that are currently being used in aquaculture.

AKAP4 as a Regulator of Human Spermatozoa

Liat Rahamim-Ben Navi, Zvi Naor
Biochemistry and Molecular Biology, Tel Aviv University, Israel

The Mitogen-activated protein kinases (MAPK) cascade is a central signaling pathway that regulates a wide variety of cellular processes, such as proliferation, differentiation, survival, apoptosis and stress responses. One of the MAPKs is ERK1/2 that plays a crucial role in signaling pathways in general and in the pituitary-gonadal axis, in particular.

We have recently reported that ERK 1/2 is a positive regulator of human spermatozoa motility and acrosome reaction. Here we describe that ERK1/2 phosphorylates A-kinase anchoring protein 4 (AKAP4), which is one of the major components of the sperm fibrous sheath and is known to be crucial for sperm motility. Furthermore, we have also found that cAMP attenuated the activation of ERK1/2 by PMA in human spermatozoa. Therefore, we decided to examine whether AKAP4 is a switch molecule that links between PKA and PKC pathways in human spermatozoa. At first we found that AKAP4 is phosphorlated by ERK1/2 in human spermatozoa and identified the phosphorylation site as threonine 265. Then we examined whether the phosphorylation of AKAP4 is important for its cellular localization. Since mature sperm do not have active transcription machinery, we used a heterologous system, i.e. in HEK293T cells expressing AKAP4. Indeed, PMA treatment led to translocation of AKAP4 from the cytosol to the Golgi in the HEK293T cells. The effect was abolished in HEK293T cells expressing AKAP4-T265A, which has a point mutation in the ERK1/2 phosphorylation site.

In order to check whether AKAP4 has a role in cAMP inhibition of ERK activation by PMA, we transfected the cells with tGFP-AKAP4, or with tGFP alone as a control. We found that cAMP and a phosphodiesterase inhibitor, IBMX decreased ERK1/2 activation by PMA in the HEK293T cells expressing AKAP4, while no inhibitory effect was noticed in the tGFP expressing cells.

Thus, aside of its known role in sperm motility, AKAP4 seems to play a role as a switch molecule that links between PKA and PKC pathways in human spermatozoa. The physiological significance of AKAP4 phosphorylation by ERK1/2 in human sperm is under investigation.

Sexual Dichotomy in Gonadal Function in Prader-Willi Syndrome (PWS): A Longitudinal Study from Early Infancy through the Fourth Decade

Harry Hirsch, Talia Eldar-Geva, Fortu Bennaroch, Yehuda Pollak, Varda Gross-Tsur

Neuropediatric Unit, Shaare Zedek Medical Center, Israel

Background: Hypogonadism in PWS is heterogeneous. Primary testicular failure is common in PWS men and variable combinations of ovarian dysfunction and gonadotropin deficiency are seen in women. Longitudinal studies focused on children, with few data for adults.

Objectives: Determine at what age the type of hypogonadism can be recognized; if there are gender specific hormonal patterns; and if these patterns are consistent throughout adulthood.

Methods: We collected 339 blood samples in 49 males (2m-36y) and 57 women (ages 1m-37y) with PWS for LH, FSH, prolactin, DHEAS, inhibin B, AMH, SHBG, testosterone (men) and estradiol (women). Duration of follow-up was 4.0±1.6 (1-6)y.

Results: In males, LH and FSH levels began to rise at ages 12-13y and 8y, respectively. Gonadotropins were normal to high in men ages 20-35y. All 14 men 20y had low testosterone (5.7±3.4 nmol/l). AMH levels decreased with age, despite low testosterone in all adult males. Inhibin B (65±58 pg/ml) was low to undetectable in most adult men. In females, LH was variable during childhood, low in 7 women 20y (0.21±0.18 mIU/ml) and normal in 6 (3.77±1.67 mIU/ml). In women ages 20 – 37y, FSH was low in 3 (0.71±0.60 mIU/ml) and normal in 10 (6.42±3.78 mIU/ml). Only 7 adult women had consistently detectable estradiol levels. AMH was below the median in most PWS females. Inhibin B was low or undetectable in all PWS females. An exaggerated rise in DHEAS was seen in some boys and girls as early as age 5y.

Conclusions: The type of hypogonadism (hypothalamic vs primary gonadal dysfunction) in PWS becomes apparent in late adolescence and early adulthood. In males, FSH levels rise from age 10y indicating progressive testicular failure. Gonadotropin levels are normal or low in PWS women. Recognition of age-related changes in reproductive hormones is important for individualizing hormone replacement and counseling.

Minichromosome Maintenance Complex Component 8 (MCM8) Gene Mutation Results in Primary Gonadal Failure

Elected as Best Clinical Abstract

Yardena Tenenbaum-Rakover[1,2], Ariella Weinberg-Shukron[3,4], Paul Renbaum[3], Dvir Dahary[5], Ephrat Levy-Lahad[3,4], Dani Bercovich[6], David Zangen[4,7]

[1]*Pediatric Endocrine Unit, Ha'Emek Medical Center, Israel*
[2]*The Rappaport Faculty of Medicine, Technion, Israel*
[3]*Medical Genetics Institute, Shaare Zedek Medical Center, Israel*
[4]*Medical School, Hebrew University, Israel*
[5]*Genetics, Toldot Ltd., Israel*
[6]*Genetic, Tel Hai College, Israel*
[7]*Pediatric Endocrinology, Hadassah Hebrew University Medical Center, Israel*

Background: Primary gonadal failure is characterized by a lack of spontaneous pubertal development, primary amenorrhea or early menopause in females, and oligospermia or azoospermia in males. Variants of the minichromosome maintenance complex component 8 gene (*MCM8*) have been recently shown to be significantly associated with women`s menopausal age in genome-wide association studies. Furthermore, *MCM8*-knockout mice are sterile. The objective of this study was to elucidate the genetic etiology of gonadal failure in two siblings from a consanguineous family presenting as primary amenorrhea in the female and as small testes and azoospermia in the male.

Methods and Results: Using whole exome sequencing, we identified a novel homozygous splice mutation (c.1954-1GA) in the recently characterized *MCM8* gene. The identified mutation segregated with the disease in the family and was absent in 100 ethnically matched control subjects. The splicing effects of the identified mutation were examined by cDNA sequencing and real-time polymerase chain reaction assays. *MCM8* cDNA sequencing in the affected individuals revealed lack of the wild-type transcript and three different aberrant transcripts predicted to result in either truncated or significantly shorter proteins. Quantitative analysis of these aberrant transcripts showed a significant decrease in MCM8 message in affected siblings homozygous for the mutation, and an intermediate decrease in heterozygous family members.

Conclusions: Our study provides first evidence of MCM8 being crucial for gonadal development and maintenance in humans, while its disruption may lead to gonadal dysgenesis. These findings open new insights into the genetic disorders of infertility and premature menopause in women.

hCG-Regulated Counter Balance Between miR-125a-3p and its Target Kinase, Fyn, in Granulosa Cells is Required for Ovulation

Hadas Grossman, Dana Chuderland, Lihi Ninio-Many, Noa Hasky, Ruth Kaplan-Kraicer, Ruth Shalgi
Cell Biology and Development, Tel-Aviv University, Israel

Ovulation is a highly coordinated process induced by LH-surge involving a vast array of signal transduction pathways. Granulosa cells support the developing oocytes and serve as transducers of the ovulatory stimulus induced by LH-surge. Fyn, a Src-family kinase, is highly expressed in the ovary and indispensable for oocyte maturation; its role in granulosa cells during ovulation was not studied before. We have recently characterized miR-125a-3p as a post-transcription regulator of Fyn in Human Embryonic Kidney 293T cells. Here, we show expression of miR-125a-3p and Fyn in mural granulosa cells of pre-ovulatory follicles, and miR-125a-3p-induced down-regulation of Fyn-expression in granulosa cells. Our aim was to explore the role of miR-125a-3p and Fyn in granulosa cells towards ovulation. We showed that administration of hCG (LH analogue) caused a 75% decrease in the *in-vivo* miR-125a-3p/Fyn-mRNA ratio; followed by a twofold increased migratory ability of mouse primary mural granulosa cells. We found that a decreased miR-125a-3p/Fyn-mRNA ratio enables hCG-induced phosphorylation of FAK and Paxillin, and increases migration of rat granulosa cell-line; suggesting a new role for mural granulosa cells in the ovulation process. An *in-vivo* interference with the miR-125a-3p/Fyn-mRNA ratio in mice granulosa cells by intra-bursal injections of Fyn-siRNA or miR-125a-3p-mimic, caused a 33% or 55% decrease in the number of ovulated oocytes, respectively; inferring the need of a proper LH-regulated miR-125a-3p/Fyn balance for ovulation. These observations reveal a new signaling network in mural granulosa cells, illustrating a counter-balance between miR-125a-3p and Fyn, under the regulation of LH/hCG. Deciphering this regulation pathway may contribute to the understanding of the pathophysiology of ovulation-related pathologies.

The Angiogenic Role of Hyaluronic Acid in Embryo Implantation

Roni Hadas[1], Aviad Cohen[1], Eran Gershon[2], Nava Dekel[1], Michal Neeman[1]
[1]*Biological Regulation, The Weizmann Institute, Israel*
[2]*Volcani Center, Agricultural Research Organization, Israel*

Implantation, a critical step in the establishment of pregnancy, is immediately followed by a marked increase in the permeability and density of the uterine blood vessels. Hyaluronic acid (HA) has been reported to participate in the regulation of vascular development in a number of physiological processes. Specifically, high molecular weight HA has been shown to inhibit angiogenesis, whereas its enzymatic degradation products are by nature pro-angiogenic. On the basis of this information we hypothesized that HA may be involved in vascular modifications associated with implantation. Our experiments revealed interesting alterations in HA distribution in the endometrium from the onset of implantation. In addition, we found an increase in HA fragmentation during early pregnancy. Moreover, substantial changes in the expression profile of genes encoding for HA synthesis and degrading enzymes, as well as in the distribution of their protein products, were observed in the implantation site during early pregnancy. Such gene and protein modifications were also noticed in the HA receptors as well as in some specific ECM stabilizing proteins. Functional MRI inspection of HA synthesis inhibitor 6-diazo-5-oxo-1-norleucine (DON)-treated pregnant mice, on embryonic day 6.5, showed a marked increase in decidual blood vessel permeability and accumulation of blood in close proximity to the implanting embryo. Moreover, MRI inspection of mice pregnant with embryos over-expressing hyaluronidase, the HA degrading enzyme, in their trophoblast cells, showed defective implantation along with increased permeability of blood vessels immediately surrounding the embryo .Taking these observations into account, we suggest that HA uterine metabolism has a pivotal role in vascular development and remodeling during embryo implantation in mice. Our study will potentially shed light on ECM participation in vascular events involved in successful pregnancy further deciphering some pathological processes responsible for implantation failure.

Characteristics of Delayed TSH Elevation in Neonatal Intensive Care Unit (NICU) Newborns in Israel

Amnon Zung[1], Arie Yehieli[2], Shlomo Almashanu[3]
[1]*Pediatric Endocrinology Unit, Kaplan Medical Center, Affiliated with the Hebrew University of Jerusalem*
[2]*Gamidor, Diagnostics*
[3]*National Center for Newborn Screening, Ministry of Health*

Background: Delayed TSH elevation (dTSH) is defined by normal TSH on the initial neonatal screening followed by elevated TSH on the second screen. For NICU newborns, the default is TSH on the first screen and T4 on the second screen. Low T4 level triggers TSH testing. Several studies concluded that dTSH is associated with low birth weight (BW), i.e. 1500 gr.

Objective: To elucidate clinical characteristics of dTSH in a large cohort of NICU newborns.

Methods: Clinical data were gathered from a cohort of 13,201 NICU newborns born between 1.1.2008 and 31.10.2014 that underwent TSH measurements due to low T4 levels on the second screen. The clinical data included gestational age (GA), BW, timing of second test and T4 levels.

Results: 333 out of 13,201 (1:40) newborns presented with dTSH (TSH 15 IU/L), 129 (39%) of them had TSH levels 40 IU/L. dTSH had a peak incidence at GA of 37-39 weeks. 66% of the patients had BW 1500 gr, and the disorder was similarly presented over the wide range of BW. TSH levels were negatively correlated with GA and BW but r-values were low. There was no optimal timing to detect the disorder, as test timing was equally distributed after the third week of life. Among 333 patients, T4 values were negatively correlated with TSH levels (R = -0.41; p 0.0001). By comparison to the healthy newborns, the 333 patients had higher BW and GA and had remarkably lower T4 levels (5.9 ± 2.8 µg/dl vs. 7.6 ± 1.7 µg/dl; p0.0001).

Conclusion: Unlike previous reports, we found that dTSH is most prevalent in full-term newborns with BW 1500 gr. Low T4 is the best predictor for this disorder, which may account for the relatively high prevalence of this disorder in our study compared to previous reports.

Incidence of Non Thyroidal Primary Malignancy (NTPM) and the association with I-131 treatment in Patients with Differentiated Thyroid Cancer (DTC).

Dania Hirsch[1,2], Alexander Gorsthein[1,2], Eyal Robenshtok[1,2], Ilan Shimon[1,2], Carlos Benbassat[1,2]

[1]*Enocrine Institute, Rabin Medical Center, Beilinson Campus, Israel*

[2]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

Introduction: The risk of Non Thyroidal Primary Malignancy (NTPM) associated with I-131 treatment (RAI) in DTC patients is an important concern. Yet, data regarding the incidence of NTPM following RAI are conflicting.

Aims: To investigate the incidence of NTPM and the association with RAI treatment in a cohort of DTC patients treated at a single tertiary medical center.

Methods: We cross matched data of 1730 patients form the Rabin Medical Center Thyroid Cancer Registry with data from the Israeli National Cancer Registry for diagnosis of NTPM. Medical records were reviewed. SPM was defined as new malignancy diagnosed at least 2 years after DTC diagnosis. Mean follow-up was 8.8 years.

Results: (a) A total of 374 NTPMs were identified in 338/1730 (19.5%) DTC patients (females 242/338, mean age 54.6±15). Of these, 143 (38.2%) were defined as SPM. (b) Of the 1355 patients included in this preliminary analysis, 1117 (82.4%) received RAI treatment and 238 (17.6%) did not. The rates of SPM in the 2 groups were 119 (10.6%) and 24 (10.1%), respectively. (c) The relative risk of SPM in DTC patients treated with RAI was not significantly increased at 1.06 (95% CI 0.7-1.6, p= 0.8) compared to untreated patients. (d) First and cumulative RAI doses in those with/without SPM were 115±46 vs 100±49 mCi and 165±135 vs 181±150 mCi, respectively. SPM rates after cumulative dose 100mCi were 49/437 (11.2%) and 65/663 (9.8%), respectively. (e) The common sites for NTPM (and SPM) were: Breast in 88 patients (40), Colon 40 (15), Melanoma 32 (10), Lymphoma 32 (8), Prostate 26 (16), Lung 20 (13), RCC 19 (8), TCC 18 (10), Non-solid tumors 12 (8).

Conclusions: NTPM in DTC patients is not uncommon and antecede DTC in most cases. Preliminary analysis of our data does not support a carcinogenic effect of RAI.

Solitary Giant Liver Metastasis from Papillary Thyroid Carcinoma – An Unusual Case of Excellent Response to Therapy

Ifat Snir[1], **Eyal Robenshtok**[2]
[1]*Liver Unit, Rabin Medical Center, Israel*
[2]*Endocrinology, Rabin Medical Center, Beilinson Hospital, Israel*

Background: Liver metastases from DTC are rare, with a reported frequency of 0.5% of distant metastases. They usually occur in the setting of widespread metastatic disease and carry a poor prognosis.

Patient findings: a 39 year old woman with a history of hypothyroidism treated with daily Levothyroxine was evaluated in the hepatology clinic due to mild elevation of GGT level that was observed during pregnancy and persisted after the birth. Abdominal ultrasound and three phasic CT demonstrated an irregular mass 78*42 mm in segment 8 of the liver. Tc-99m labeled RBC SPECT was negative. Liver biopsy was performed and revealed morphological and immune-histochemical feature that were compatible with differentiated thyroid cancer. Neck US demonstrated three suspicious lesions in the left lobe of the thyroid, 3, 4, 5 mm size, but several attempts to perform FNA from the lesions were non-diagnostic. Systemic evaluation was negative for other metastasis. The patients underwent partial liver resection with free surgical margins, which demonstrated a solitary 7.5cm metastasis compatible with DTC. Next, the patient underwent total thyroidectomy which demonstrated multifocal bilateral follicular variant papillary thyroid carcinoma ranging from 0.1 cm to 0.5cm with minimal extra-thyroidal extension. Following the two surgeries suppressed thyroglobulin (Tg) was 0.8 with negative antibodies. Postoperatively, thyroid radioiodine ablation was performed with 188 mCi, followed by whole body scan showing uptake only in the thyroid bed. Suppressive treatment with levothyroxine was prescribed, and the patient was followed with neck ultrasound, liver MRI and suppressed Tg levels. Stimulated Tg was evaluated 12 month after the RAI ablation using recombinant human TSH (Thyrogen™) and was found to be less than 0.2ng/ml. The combination of negative imaging studies and an undetectable stimulated Tg levels define the patient as excellent response to therapy, which carries a favorable long term prognosis.

Summary: Metastatic liver involvement from DTC is nearly always multiple or diffuse and is usually found along with other distant metastases. We describe for a rare case of giant solitary liver metastasis which was successfully treated with surgery and radioiodine. Patients with limited liver involvement from DCT should be treated with surgery and radioiodine with curative intent.

Subclinical Thyroid Disease Increases all-cause Mortality in the Elderly

Alon Grossman[1,5], Avraham Weiss[2,5], Nira Koren-Morag[3], Ilan Shimon[1,5], Yichayahou Beloosesky[2,5], **Joseph Meyerovitch**[4,5]

[1]*Unit of Endocrinology and Metabolism, Rabin Medical Center, Beilinson Campus, Israel*

[2]*Department Of Geriatrics, Rabin Medical Center, Beilinson Campus, Israel*

[3]*Department of Epidemiology and Preventive Medicine, Sackler Faculty of Medicine, Tel Aviv University, Israel*

[4]*Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel, Israel*

[5]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

Importance: The association between subclinical hypothyroidism (scH) and hyperthyroidism (scHy) and mortality in the elderly is poorly defined.

Objective: To evaluate the association between scH and scHy and mortality in the elderly and to define the thyroid stimulating hormone (TSH) values associated with excess mortality in the elderly

Methods: Review of a computerized database of a large health care organization. Patients older than 65 years evaluated in the years 2002-2012 with documented normal free T4 values were included in the analysis. All cases of known thyroid disease or cases in which thyroid medications were dispensed were excluded. Subjects were divided into 3 groups based on TSH values: normal (normal TSH), scH (TSH 4.2 mIU/L) and scHy (TSH

Setting: Ambulatory patients treated by a large health care organization (Clalit Health Medical Organization).

Participants: Patients older than 65 years evaluated in the years 2002-2012 with documented normal free T4 values

Main Outcome and measures: All-Cause mortality according to TSH values.

Results: A final analysis was performed on 17,440 individuals with subclinical thyroid disease {538 scHy (3.1%), 1956 scH (11.2%), normal 14,946 (85.7%) average age 83±6.5 years} who were followed-up for 10 years. Both scH (HR= 1.75 CI 1.63-1.88) and scHy (HR= 2.33 CI 2.08-2.63) were associated with significantly increased mortality that persisted on multivariate analysis for age, gender, chronic kidney disease, chronic obstructive lung disease, smoking, dementia, Parkinson`s disease, cerebrovascular disease, congestive heart failure, diabetes mellitus and hypertension (scH HR=1.68 CI 1.56-1.8, ScHy HR=1.93 CI 1.7- 2.17). TSH values 6.35 mIU/L were associated with the highest mortality in those with scH, whereas in scHy, no threshold for increased mortality was identified.

Conclusions and relevance: Both scH and scHy are associated with increased mortality in the elderly. A threshold TSH value (6.35 mIU/L) exists for increased mortality in scH, but not in scHy. This should be taken into account when considering treatment in elderly individuals with subclinical thyroid disease.

Towards An Optimal Viral Vector with Toxic Cargo Targetting Thyroid Carcinoma Cells: In Vitro Studies in Human Thyroid Cancerous Cells Harvested During Surgery

Nathan Landis[1,4], Nataly Urshanski[1], Meital Grafi-Cohen[1], Ettie Knoll[1], Elena Izkhakov[1,4], Zaki Kraiem[1], Asaf Aizic[3], Gary Weisinger[1], Moshe Yehuda[2,4], Dan Fliss[2,4], Naftali Stern[1,4]

[1]*Institute of Endocrinology, Metabolism and Hypertension, Sourasky Medical Centre, Israel*

[2]*Head and Neck Surgery, Sourasky Medical Centre, Israel*

[3]*Department of Pathology, Sourasky Medical Centre, Israel*

[4]*Sackler Faculty of Medicine, Tel-Aviv University, Israel*

Thyroid carcinoma comprises ~2% of all tumours, but 90% of endocrine-related tumours. Anaplastic thyroid carcinomas, as well as some differentiated thyroid cancers, may have poor outcome, emphasising the need for new therapeutic modalities. Identification of adeno-associated viruses (AAV), with a unique integrated promoter-enhancer sequence and a preferred "suicide" gene cargo has proven to be an effective tool in gene therapy experimentally and is currently undergoing clinical evaluation. The present work integrates the outcome of three subprojects: a. construction of a thyroid-specific thyroglobulin promoter/enhancer; b. selection of the most suitable AAV serotype in terms of thyroid cancer cell infectivity; c. selection of an optimised toxic cargo to be inserted into and borne by the selected virus. Based on results of in vitro GFP-based infection rates in thyroid carcinomas from 4 patients, we selected the optimal AAV-DJ serotype. Diphtheria (DT) and ganciclovir-inducible herpes-thymidine kinase (TK) toxins were selected, based on past experience. The toxins' sequences were added to cargo plasmids carrying a unique thyroid-optimised thyroglobulin-based promoter/enhancer unit developed in our lab. With the thyroid-optimised herpes-TK cargo, and concentrations of ganciclovir at 20 µg/ml/day, AAV-DJ caused 99% cell death in a cultured primary human follicular carcinoma and 19% in its matched normal thyroid cells; 90% in a papillary carcinoma and 19% in its surrounding normal cells; and 24% in another papillary carcinoma. In control vascular smooth muscle cells, at this concentration of ganciclovir, no significant cell death was detected. Thyroid-optimised diphtheria toxin caused 50% cell death in the follicular carcinoma, and 45% in the papillary carcinoma. In a single batch of non-cancerous thyroid cells thus far tested, 9% cell death was observed. Based on current results, TK, supplemented by ganciclovir for activation, is the better toxin due to higher potency in killing the thyroid carcinomas. Future work will involve testing more patient samples to confirm selectivity of both treatments, and altering the viral capsid to optimise treatment against thyroid cancer, thus completing the "ideal" treatment paradigm.

A Sorafenib Sparing Effect in the Treatment of Thyroid Carcinoma Attained by Co-Treatment with the Novel Isoflavone Derivative (cD-tboc) and 1,25 Dihydroxyvitamin D (1.25D)

Elena Izkhakov[1,4], Orli Sharon[1], Esther Knoll[1], Asaf Aizic[2,4], Dan Fliss[3,4], Rona Limor[1,4], Naftali Stern[1,4], Dalia Somjen[1,4]

[1]*Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center, Israel*

[2]*Istitute of Pathology, Tel Aviv Sourasky Medical Center, Israel*

[3]*Ear, Nose, Throat, Head and Neck Surgery Department, Tel Aviv Sourasky Medical Center, Israel*

[4]*Sackler Faculty of Medicine, Tel Aviv University, Israel*

Background: Sorafenib is aproved for the treatment of patients with progressive radioactive iodine-refractory differentiated thyroid cancer (DTC). Sorafenib significantly improves progression-free survival, but causes severe side effects.

Estrogens may enhance thyroid cancer cell growth. Our group has recently reported that a novel anti-estrogenic compound cD-tboc retards growth of both human thyroid carcinoma cell lines and cultured human carcinoma cells. Vitamin D receptor (VDR) is expressed in malignant dividing cells and responds to 1.25D by decreased proliferative activity *in vitro*.

Aims: To evaluate the effects of different doses of sorafenib alone and in combination with cD-tboc and 1.25D on cell proliferation (CP) in cultured human papillary thyroid carcinoma (PTC) compared to cultured normal thyroid cells.

Methods: CP was determined by direct measurement in 8 cultured PTC and normal thyroid specimens harvested during thyroidectomy from the same patients.

Results: Sorafenib inhibited CP dose-dependently, and cancer cells were more sensitive than normal cells to this inhibitory activity. Combined treatment with sorafenib (200 µg/ml) with low concentration of cD-tboc (0.3 µM) reduced CP in cancer cells, whereas this effect was much smaller in normal thyroid cells. Most importantly, the inhibitory effect of sorafenib on CP in cancer cells was amplified after addition of cD-tboc (0.3 µM) and 1.25D (50 nM); so that not only was the maximal inhibition larger (-53% vs. -61% P

Conclusions: This is the first report that low concentration cD-tboc and 1.25D markedly amplifies the inhibitory effect of sorafenib and allows the use of a 10 fold lower concentration sorafenib on the growth of human PTC derived from patients. This finding might form the basis for the use of a new combined treatment for progressive radioactive iodine-refractory DTC.

In Euthyroid Humans Younger than 40 years, TSH Enhances Thyroxine(T4) Conversion to Triiodothyronine(T3).

David Strich[1], Gilad Karavani[2], Shalom Edri[3], David Gillis[2]
[1]*Pediatrics, Sharre Zedek Medical Center and Clalit health Service Jerusalem District*
[2]*Pediatrics, Hadassah Medical Center*
[3]*Data Center, Clalit Health Service Jerusalem District*

Introduction: About 80% of T3 production is due to extra thyroidal conversion of T4 to T3 by two deiodinases (D1 and D2). TSH has been shown to enhance deiodinase activity in thyroid cell cultures. There is clinical evidence that this effect is relevant in-vivo in children. It is not known if this effect is significant in adults.

Methods: free T3 (FT3), free T4 (FT4) and TSH levels from 861,475 sera taken from patients age 1 year or greater were studied. Initial exclusions were: missing data for either TSH or one of the thyroid hormones and TSH greater than 7.5 mIU/l. Samples from patients taking, or who had taken, thyroid medications or/and drugs that may interfere with thyroid hormone activity were then removed by crossing data from electronic patient files. The 27,940 samples that remained after all exclusions were stratified by age and analyzed in order to investigate relations between TSH, FT4 and FT3 in the euthyroid or near-euthyroid state.

Results: For each increasing TSH quartile, FT3 and the FT3/FT4 ratio increased and FT4 decreased significantly (for both FT3, FT4 and FT3/FT4 ratio, p

Conclusion: Increasing TSH levels are expected to be higher as FT4 and FT3 levels decrease. Surprisingly, within the euthyroid range, as TSH increases FT3 levels increase but FT4 levels decrease. This phenomenon occurs only in the young (below 40) age groups and disappears later. The change in these relations may reflect a decrease in T4 to T3 conversion with age, which may be one component of the ageing process.

Disorders of the Calcium Sensing Receptor

Auryan Szalat
Endocrine Institute and Metabolism Unit,, Hadassah Ein-Keren Hospital, Jerusalem

The Calcium-Sensing receptor (CaSR) was identified two decades ago, and is today recognized as playing a critical role in calcium homeostasis. CaSR gene, localized on the chromosome 3q13.3-21 encodes a protein structurally affiliated to the G-protein coupled receptors superfamily. It regulates the secretion of parathyroid hormone and renal calcium excretion by inducing a cascade of intracellular signals leading to changes in intracellular inositol 1,4,5-triphosphate and calcium concentrations, as well as phosphorylation of mitogen-activated phosphate kinases.
Patients with CaSR-related diseases have a typical phenotype characterized by a discrepancy between blood calcium levels and urinary fraction excretion of calcium (uFeCa), due to either gain-of-function (GOF) or loss-of-function (LOF) of the CaSR.
CaSR may be a cause of parathyroid disease by three possible mechanisms: (1) genetic autosomal dominant LOF (Familial Hypocalciuric Hypercalcemia (FHH), Neonatal Severe Primary Hyperparathyroidism) or GOF (Autosomal Dominant Hypocalcemic Hypercalciuria (ADHH)) mutations are identified in the spectrum of familial disease. (2) Functional auto-antibodies to CaSR. (3) Genetic mutations in other genes on chromosome 19p or 19q13 encoding proteins regulating CaSR activity as the G-protein subunit alpha11 and the Adaptor protein-2 σ subunit.
Clinicians evaluating patients with parathyroid disorders should identify the ones who may have a CaSR-related disease and the other who probably do not. Based on a review of the literature and our own experience, the following 10 points-to remember can be helpful: (1) autosomal dominant pattern of familial parathyroid disease with appropriate blood and urine calcium levels discrepancy is the straightforward and simplest clinical situation. However there are many potential pitfalls: (2) de-novo mutations can occult the familial pattern of the disease. (3) primary hyperparathyroidism is often associated with low 25 hydroxy-vitamin D levels and can mimic FHH phenotype; (4) sometimes, association of parathyroid adenoma and FHH can be found. (5) Patients with hypoparathyroidism treated with oral calcium and vitamin D will develop hypercalciuria, which can mimic ADHH; thus, uFeCa before starting any calcium-vitamin D supplementation is critical in such patients in the aim to identify those with pre-therapeutic high index of suspicion for CaSR disorder. (6) In the evaluation of patients with suspected FHH, controversies exist regarding the appropriate uFeCa cut-off: 1% may be more specific, but 2% more sensitive. (7) Despite oriented CaSR genetic evaluation, more than 20% of patients with typical FHH phenotype will have normal sequencing. (8) Patients with CaSR antibodies usually have an auto-immune background disease, and should be diagnosed by laboratory methods as ELISA or FACS. (9) Identification of a positive genetic finding or CaSR antibodies does not prove a causal relationship until a positive functional study is performed based on evaluation of intracellular signaling as exposed earlier. (10) Patients with familial disease or typical phenotype suggesting CaSR-related disease but with normal evaluation, should be referred for further explorations, as probably other factors regulating CaSR activity are still unknown.

Effect of Meal Timing on Daily Metabolic Rhythms and Body Weight

Oren Froy
Institute of Biochemistry, Food Science and Nutrition, The Hebrew University of Jerusalem

Disruption of circadian rhythms leads to obesity and metabolic disorders. Time restricted feeding (RF) provides a time cue and resets the circadian clock leading to better health. In contrast, high-fat (HF) diet leads to disrupted circadian expression of metabolic factors and obesity. We tested whether long-term clock resetting by RF can attenuate the disruptive effects of diet-induced obesity. Analyses included liver clock gene expression, locomotor activity, blood glucose, metabolic markers, lipids and hormones around the circadian cycle for a more accurate assessment. Compared with mice fed HF diet ad libitum, timed HF diet restored the expression phase of some clock genes and phase-advanced others. Although timed HF-fed mice consumed the same amount of calories as ad libitum low fat-fed mice, they showed reduced body weight, reduced cholesterol levels and increased insulin sensitivity. Compared with mice fed HF diet ad libitum, timed HF diet led to lower body weight, decreased cholesterol levels, reduced TNFα levels and improved insulin sensitivity. Timed HF-fed mice exhibited a better-satiated and less stressed phenotype of lower ghrelin and corticosterone levels compared with mice fed timed low-fat diet. Altogether, our findings suggest that timing can prevent obesity and rectify the harmful effects of HF diet. Our clinical studies in humans support these findings.

The Role of the Islet Micro-environment in Beta-cell Function

Limor Landsman
Faculty of Medicine, Tel Aviv University

Type 2 diabetes arises when the endocrine pancreas fails to secrete sufficient insulin to cope with the metabolic demand, because of beta-cell dysfunction and/or decreased beta-cell mass. Using novel mouse systems, our lab studies how adult beta-cell function is maintained in heath, and why it is lost in diabetes. To this end, we focus on the role of the pancreatic mesenchyme, a central component of the islet microenvironment. Our observations point to a previously unappreciated role of these cells in glucose homeostasis. Our findings therefore suggest the pancreatic mesenchyme can serve as a novel target for anti diabetes therapies.

Recent Advances in the Pathogenesis of Primary Aldosteronism

Silvia Monticone

Division of Internal Medicine 4 and Hypertension Unit, Department of Medical Sciences, University of Torino. Torino, Italy

Primary aldosteronism (PA) is a heterogeneous group of disorders, comprising both sporadic (aldosterone producing adenoma – APA – and bilateral adrenal hyperplasia – BAH –) and familial forms, named as Familial hyperaldosteronism type I, type II and type III (FH-I, FH-II and FH-III). Classically, PA was considered to be a rare and relatively benign form of secondary hypertension, accounting for less than 2% of the hypertensive population. However, over the last years, the wide application of the aldosterone/plasma renin activity ratio as screening test has dramatically increased the rate of diagnosis of PA, which is now recognized as the most frequent form of endocrine hypertension. Similarly, it has been extensively demonstrated that aldosterone "per se" causes an increase in cardio-vascular risk, which is at least in part independent from its effect on blood pressure.

Despite significant progress in the diagnosis and management of PA patients, until recently, the only subtype of PA whose underlying genetic and molecular basis was clearly understood was FH-I. The introduction of next-generation sequencing (NGS) has had a profound impact in our understanding of the molecular determinants of PA. In particular, the use of NGS has led to the identification of somatic mutations in several genes (*KCNJ5*, *ATP1A1*, *ATP2B3* and *CACNA1D*) responsible for the autonomous aldosterone overproduction in APAs. The mutations, through different mechanisms, lead to a an increase in intracellular calcium concentration, which is central for the regulation of aldosterone production, by increasing the transcription of *CYP11B2* (aldosterone synthase). Of note, germline mutations in the *KCNJ5* gene, encoding the inward rectifying K^+ channel GIRK4, represent the molecular basis of FH-III, a rare and particularly severe form of FH, often requiring bilateral adrenalectomy to achieve blood pressure control. Similarly, de novo *CACNA1D* germline mutations have been described in two children presenting with early onset PA and neurological developmental disorders.

Plenary Lecture 3:

Half a Century and a Bit in Diabetes

Erol Cerasi
Endocrinology Department, The Hebrew University of Jerusalem

I will present a brief history of my career: born, raised and trained in Istanbul, re-trained in Stockholm where I initiated my scientific career, established in Jerusalem where I created the endocrinology department of Hadassah and established a diabetes research laboratory, I stubbornly focused my work throughout half a century on insulin secretion and beta cell biology. I have maintained, since the very early 1960's, that deficient beta cell function is at the root of type 2 diabetes, and this against the majority of scientists and clinicians over decades. I notice with deep satisfaction that this is now the consensus view. I have published more than 300 papers, but at my burial I will take with me no more than some 30 of them; I will briefly delineate the topics by which I would like to be judged.

Follow-Up of Newborns of Mothers with Graves' Disease

Yael Levy-Shraga,[1,2] Liran Tamir-Hostovsky,[1,2] Valentina Boyko,[3] Liat Lerner-Geva,[3] and Orit Pinhas-Hamiel[1,2]

[1]Pediatric Endocrine and Diabetes Unit, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Ramat Gan, Israel.

[2]Sackler School of Medicine, Tel-Aviv University, Tel Aviv, Israel.

[3]The Women and Children's Health Research Unit, Gertner Institute, Ramat Gan, Israel.

Background: Overt neonatal Graves' disease is rare, but may be severe, even life threatening, with deleterious effects on neural development. The main objective of this study was to describe the course of thyrotropin (TSH) and free thyroxin (fT4) levels, as well as postnatal weight gain in relation to fT4 levels, in neonates born to women with Graves' disease without overt neonatal thyrotoxicosis. Such information is important to deduce the optimal schedule for evaluation.

Methods: We conducted a retrospective chart review of neonates born to mothers with Graves' disease between January 2007 and December 2012. The records were reviewed for sex, gestational age, birth weight, maternal treatment during pregnancy, and maternal thyroid stimulating immunoglobulin (TSI) level. For each visit in the clinic, the data included growth parameters, presence of symptoms suspected for hyperthyroidism, blood test results (levels of TSH, fT4, and TSI), and treatment.

Results: Ninety-six neonates were included in the study (49 males), with a total of 320 measurements of thyroid function tests (TSH and fT4). Four neonates (4%) had overt neonatal Graves' disease; one of them along with nine others were born preterm. In 77 (92.9%) of the remaining 83 neonates (the subclinical group), fT4 levels were above the 95th percentile on day 5. All had normal fT4 on day 15. A negative association was found between fT4 and weight gain during the first two weeks.

Conclusions: In this cohort, most neonates born to mothers with Graves' disease had a subclinical course with abnormal fT4 levels that peaked at day 5. After day 14, all measurements of fT4 returned to the normal range, although measurements of TSH remained suppressed for up to three months. Elevated fT4 was associated with poor weight gain.

Is Every Disease Metabolic?- Metabolic Regulation of Behavior and Cognition

Ayelet Erez
Weizmann Institute of Science

Metabolic rewiring accompanies systemic disorders and vice versa, metabolic disorders can have systemic manifestations. Argininosuccinic aciduria (ASA) is a urea cycle disorder (UCD) caused by deficiency of argininosuccinic lyase (ASL). In spite of diagnosis by newborn screening and early initiation of therapy, patients with ASA have a higher incidence of neurocognitive abnormalities as compared to subjects with other UCDS, even in the absence of significant hyperammonemia. The mechanisms underlying this paradoxical observation are yet unclear. Recently, we reported that ASL is essential for the utilization of arginine for synthesis of nitric oxide (NO) and that NO deficiency could explain some of the unique features such as hypertension in ASA. As NO has an established role in neurogenesis and neurotransmission, we hypothesized that NO deficiency in the brain contributes at least in part to the cognitive delays in ASA.

To further understand the importance of ASL/NO in neuronal function, we assayed the expression of ASL in wild-type mouse brains. Our preliminary results show that deleting Asl in the brain stem of mice by using a virus cre cause a dramatic phenotype with hyperactivity that correlated with increased catecholamine levels especially norepinephrine in the plasma of the injected mice. Since decreased NO has been associated with increased sympathetic outflow from the brainstem, our results suggest that disruption of non-ureagenic functions of ASL may underlie the neurocognitive deficits in ASA. Corroborating these animal studies, our analysis of data from the longitudinal study of UCDs, show that in the absence of hyperammonemia, patients with ASA have significantly increased self-reported short attention span as compared to those with other urea cycle disorders (48.7 vs 18%; $p<0.05$).

Our preliminary results support NO deficiency as a contributor for the increased neurocognitive delay observed in ASA and have clinical implications relevant to optimization of the current treatment modalities for ASA. More broadly, our results shed light on the role of ASL and NO in neurobehavior and cognition.

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

R

S

T

U

V

W

Y

Z