

**THE 36th ANNUAL MEETING OF
THE ISRAEL ENDOCRINE SOCIETY
17-18 APRIL 2007, KFAR HAMACCABIA, RAMAT-GAN**

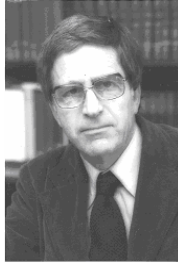
Tuesday, April 17, 2007

- 07:45 **Registration and poster hanging**
- 09:00 **Three parallel free communication sessions (FC)**
- FC1 (A1-A5) DIABETES, INSULIN RESISTANCE AND OBESITY** ▶ Rayman A
- FC2 (A6-A10) PUBERTY, GROWTH, AND SEXUAL DEVELOPMENT** ▶ Rayman C
- FC3 (A11-A15) HORMONES AND CANCER** ▶ Rakefet
- 10:15 **Coffee Break and Poster Session (P48-P91)**
- THYROID/ADRENAL/ BONE, PUBERTY/GROWTH/GH/ IGF1
/NEUROENDOCRINOLOGY, DIABETES, OBESITY &
ATHEROSCLEROSIS, CANCER, SIGNALING,
REPRODUCTION**
- PL1 PLENARY LECTURE** ▶ Rayman B
- 11:15 **Genetics of Thyroid Cancer Guides Design of Novel
Therapeutic Strategies** - Prof. James A. Fagin, *Memorial Sloan-
Kettering Cancer Center, New York, NY*
- 12:10 **IES - BUSINESS MEETING**
- 12:45 **Lunch**
- 13:45 **Three parallel free communication sessions (FC)**
- FC4 (A16-A20) ENDOCRINE PANCREAS, INSULIN SECRETION AND
SIGNALING** ▶ Rayman A
- FC5 (A21-A25) ENDOCRINE CANCER** ▶ Rayman C
- FC6 (A26-A30) ENDOCRINOLOGY OF REPRODUCTION** ▶ Rakefet
- 15:00 **Coffee Break**
- SL Two Parallel Symposia**
- SL1 Israeli Endocrine Society Guidelines for Thyroid Cancer** ▶ Rayman A
- 15:15 S1 **Israeli Guidelines for Thyroid Cancer** – Dr. Jonathan Arbelle
Maccabi Sherutey Briut, Beer-Sheva
- 16:00 S2 **Case presentations** – Dr. Carlos Benbassat, *Rabin Medical
Center, Petah-Tiqva*
- 16:20 S3 **Open Discussion**
- SL2 Comparative Endocrinology** ▶ Rayman C
- 15:15 S5 **The Effect of Leptin and GnRh on the Reproduction of
Tilapia as a Model Fish** - Dr. Bertha Sivan, *Department of
Animal Science, Faculty of Agriculture, Hebrew University of
Jerusalem, Rehovot*
- 15:40 S6 **The Role of Retina and Extra Retinal Photo Stimulation in
Reproductive Activities of Domestic Birds** - Dr. Israel
Rosenboim, *Department of Animal Science, Faculty of Agriculture,
Hebrew University of Jerusalem, Rehovot*
- 16:05 S7 **Multi-Level Regulation of Milk Protein Synthesis** - Dr.
Itamar Barash, *Institute of Animal Science, Agricultural Research
Organization, the Volcani Center, Bet-Dagan*
- 16:30 S8 **Prolactin, Leptin and The Mammary Gland** - Dr Avi
Shamay, *Institute of Animal Science, Agricultural Research
Organization, the Volcani Center, Bet-Dagan*

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Wednesday, April 18, 2007

- 07:45 **Registration (for those not registered on the 1st day) and poster hanging**
- 08:30 **Three parallel free communication sessions (FC)**
- FC7 (A31-A36) BONE METABOLISM AND VITAMIN D** ▶ Rayman A
- FC8 (A37-A41) ADRENAL** ▶ Rayman C
- FC9 (A42-A47) INTRACELLULAR SIGNALING MECHANISMS** ▶ Rakefet
- 10:00 **Coffee Break and Poster session**
- SL3 Endocannabinoid System (Sponsored by Sanofi-Aventis)** ▶ Rayman B
- 10:45 **The Endocannabinoid System: an Overview of its Physiological Roles** - Prof. Raphael Mechoulam, *The Hebrew University of Jerusalem*
- 11:20 **The Role of the Endocannabinoid System in Common Obesity** - Dr. Gabriella Lieberman, *Sheba Medical Center, Tel-Hashomer*
- 11:45 **Endocannabinoid Regulation of Bone Remodeling** - Prof. Itai Bab, *The Hebrew University of Jerusalem*
- PRIZE LECTURES AND PRIZE ANNOUNCEMENTS** ▶ Rayman B
- 12:15 **Recognition Award for Special Contribution to Endocrinology in Israel – to Prof. Zvi. Laron**
- 12:25 **LINDNER PRIZE – In vitro Models of Human Pituitary Hormone Regulation** - Dr. Ilan Shimon, *Rabin Medical Center, Petah Tiqva.*
- 12:55 **CHOWERS PRIZE – A Servant to two Masters: Tissue-Specific Regulation of Steroidogenic P450 (Cyp11a) in the Ephemeral Placenta and Cycling Ovary** - Noa Sher, *The Hebrew University of Jerusalem*
- Low Expression of COX-2, Reduced Cumulus Expansion, and Impaired Ovulation in SULT1E1-deficient mice** - Dr. Eran Gershon, *Biological Regulation, Weizmann Inst., Rehovot*
- 13:25 **Announcement of Best Clinical and Basic Abstracts; Best Posters**
- 13:40 **Lunch**
- SL4 Endocrine-related Cancer (Sponsored by Novartis)** ▶ Rayman A
- 14:45 **S9 Prostate Cancer: A Disease of Ectopic Steroidogenesis Regulated by Insulin-like Growth Factors** - Prof. Steve Plymate, *Univ. of Washington, Seattle, WA*
- 15:15 **S10 Breast Cancer: Endocrine and Genetic Risk Factors - an Update** - Prof. Eitan Friedman, *Sheba Medical Center, Tel-Hashomer*
- 15:45 **S11 Obesity and Cancer: Novel Molecular Mediators** - Dr. Hana Kanety, *Sheba Medical Center, Tel-Hashomer*
- 16:15 **S12 Dietary Intervention in Steroid Hormone Action** - Prof. Yoav Sharoni, *Ben-Gurion Univ. of the Negev, Beer-Sheva*
- 17:00 **Closure**



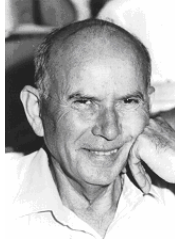
פרופ' הנס יוחנן לינדנר ז"ל

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 קיימברידג' שבאנגליה. עם תום לימודיו, חזר לינדנר לאוסטרליה, התמנה כחוקר בכיר ב-Commonwealth Scientific Research Organization והתרכז בחקר פיטואסטרונגים. 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 מולטידיסציפלינארית 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 לוטרינריה ואף היה נשיא האגודה הישראלית לאנדוקרינולוגיה. בתקופת כהונתו החלה מסורת קיום הכנסים השנתיים. פרופ' לינדנר היה פעיל גם בארגונים בינלאומיים: חבר בוועדות של WHO, של מכון מקס-פלאנק בגרמניה, של INSERM בצרפת, של ארגונים אנדוקריניים בינלאומיים 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 לאנדוקרינולוגיה. 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- חיים ורנר
- 2002- משה פיליפ
- 2003- שרה פרבר
- 2004- פואד פארס
- 2006- איתן גרוס
- 2007- אילן שמעון



פרופ' ישראל חוברס ז"ל

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 אזור 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 ובניורולוגיה, החל פרופ' חוברס להתעניין באנדוקרינולוגיה ואף היה בין הראשונים שקיבל תואר רופא מומחה בשטח זה בארץ. הוא התעניין במיוחד בתחום הניורואנדוקרינולוגיה שבו תרם רבות מבחינה עיונית ומחקרית.

בשנת 1962 יצא פרופ' חוברס מטעם NIH להשתלמות באוניברסיטה של פנסילבניה, שם עבד בשיתוף עם פרופ' McCann שעבודתו הקנתה לו מעמד של חלוץ במחקר הניורואנדוקריני בתחום הקשר בין ההיפותלמוס והורמוני יותרת המוח, ובעיקר בגילוי ובאפיון של הפקטור ההיפותלמי המזרז את הפרשת הגונדוטרופינים מיותרת 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 לאנדוקרינולוגיה ניסויית במסגרת מחלקת עצבים.

פרופ' חוברס וקבוצתו עסקו בחקר מנגנונים עצביים ואנדוקריניים הקשורים בווסות חום הגוף ובתפקיד מערכת העצבים המרכזית בווסות 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 הניורואנדוקרינולוגיה. 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 לאוכלוסייה 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 בפרוזה'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 | 1997 - פואד פארס | 2002- רינה המי |
| 1993- טלי נוה-מני | 1998 - אסף רודיך | 2003 - יעל קלמה |
| 1994- ליאורה שוקובסקי | 1999- סיגל כורם | 2004 - שלומי לזר |
| 1995- איריס קרן-טל | 2000 - אפרת וורטהיים | 2006- אמיר תירוש |
| 1996- קרן פז | 2001 - אלון חן | 2007- נועה שר וערן גרשון |

Tuesday, April 17, 2007

- 07:45 **Registration and poster hanging**
- 09:00 **Three parallel free communication sessions (FC)**
- FC1 (A1-A5) DIABETES, INSULIN RESISTANCE AND OBESITY**
Chair: S.R. Sampson, M. Walker
- 09:00 A1 **Role of p38-MAPK and ErbB receptors pathways in the induction of IRS-1 serine phosphorylation and insulin resistance by TNF**
Yafit Rachminov, Ehud Barhurdar, Uri Nir, Maayan Barnea, Zecharia Madar, Avi Karasik, Rina Hemi, Hannah Kanety
The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University; Institute of Endocrinology, Sheba Medical Center; Faculty of Agriculture, The Hebrew University of Jerusalem
- 09:15 A2 **Cytosolic Protein Tyrosine Phosphatase epsilon (cytPTPe) is a Negative Regulator of Insulin Signaling in Skeletal Muscle.**
Shlomit Aga-Mizrachi, Avraham I. Jacob, Tamar Brutman-Barazani, Asia Bak, Ari Elson, Sanford R. Sampson
Bar-Ilan University, Ramat-Gan; Weizmann Institute of Science, Rehovot
- 09:30 A3 **Crosstalk between inflammation and insulin resistance in insulin target tissues: NFkB represses GLUT4 gene promoter.**
Shiri Karni, Michal Armoni, Chava Harel, Simcha Milo, Eddy Karnieli
Techion- Israel Institute of Technology; Rambam Medical Center
- 09:45 A4 **Identification and Characterization of Novel Adiponectin Receptor 1 Isoforms**
Rina Hemi, Reut Ashwall, Reut Rosenblum, Avi Karasik, Hannah Kanety
Institute of Endocrinology, Sheba Medical Center; The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University
- 10:00 A5 **Regulation of the gene encoding GPR40, a potential missing link between obesity and diabetes**
IES PRIZE FOR BEST ABSTRACT IN BASIC RESEARCH
Gabriela Ridner, Reut Bartoov-Shifman, Keren Bahar, Michael D. Walker
Weizmann Institute of Science-Rehovot
- FC2 (A6-A10) PUBERTY, GROWTH, AND SEXUAL DEVELOPMENT**
Chair: E. Hershkovitz, L. Lazar
- 09:00 A6 **Evidence that prenatal maternal serotonin reuptake inhibitors (SSRIs) therapy induce changes in anthropometrical parameters and in the hormonal profile of newborns**
Diana Prokonov, Irit Gil-Ad, Rachel Maayan, Amichai Zolokov, Abraham Weizman, Shmuel Davidson
Department of Neonatology Rabin Medical Center; Lab Biological Psychiatry, Felsenstein Medical research Center Tel-Aviv University
- 09:15 A7 **IGF-I Replacement Therapy in Children with Congenital IGF-I Deficiency Restore Heart Dimension and Function in Children with Laron Syndrome**
Zvi Laron, Olga Kisselgoff, David M. Steinberg, Yzhar Toren, Miche Feinberg, Mickey Scheinowitz
Endocrinology and Diabetes Research Unit and Cardiac Clinic, Schneider Children's Medical Center, Petah-Tikva; Faculty of Exact Sciences, Tel-Aviv University; The Heart Institute, Sheba Medical center and Neufeld Cardiac Research Institute, Tel-Aviv University
- 09:30 A8 **11 β HSD-1 activity in the programming of growth retardation and metabolic morbidity in SGA**
Nehama Zuckerman-Levin, Larisa Ziblin, Carlos Knopf, Oshrat Baruch, Ze'ev Hochberg
Pediatric Endocrinology, Meyer's Childrens Hospital, and Clinical Biochemistry, Rambam Medical Center, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa
- 09:45 A9 **Neuroendocrine Phenotype Analysis in Five Patients with Isolated Hypogonadotropic Hypogonadism Due to a L102P Inactivating Mutation of GPR54**
Yardena Tenenbaum-Rakover, Monique Commenges-Ducos, Andriovane, Chantal Aumas, Osnat Admoni, Nicolas de Roux
Pediatric Endocrine Unit, Ha'Emek Medical Center, Afula, Technion Faculty of Medicine, Haifa, Israel

Maternité Pellegrin, CHU Bordeaux, 33076 Bordeaux, INSERM U690.
Hopital Robert Debré, 75019 Paris, Faculté de Médecine Paris Sud, 94275
Le Kremlin Bicêtre, France

- 10:00 A10 **Endocrine care of transsexual men in Israel: a longitudinal study**
Yona Greenman, Naftali Stern.
Institute of Endocrinology and Metabolism, Tel Aviv-Sourasky Medical Center, Tel Aviv

FC3 (A11-A15) HORMONES AND CANCER

Chair: R. Koren, Y. Sharoni

- 09:00 A11 **Molecular mechanisms for activation of the antioxidant response element transcription system by dietary compounds**
Hagar Salman, Yaara Amosi, Michael Danilenko, Joseph Levy, Yoav Sharoni
Faculty of Health Sciences, Ben-Gurion University of the Negev and Soroka Medical Center of Kupat Holim, Beer-Sheva,
- 09:15 A12 **Interactions between androgen receptor and BRCA1 in regulation of IGF-I receptor gene expression in prostate cancer cells**
Hagit Schayek, Kathy Haugk, Lawrence True, Stephen R. Plymate, Haim Werner
Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv; Departments of Pathology and Medicine, University of Washington, Seattle, Veterans Administration Puget Sound Health Care System-Geriatric Research Education and Clinical Center, Seattle
- 09:30 A13 **Impaired Insulin Signaling is Associated with Decreased Incidence of Experimental Skin Cancer.**
Jenny Russ, Weingarten Galina, Junji Takeda, C. Ronald Kahn, Efrat Wertheimer
Department of Pathology, Sackler Faculty of Medicine, Tel Aviv University, Israel. Dermatology, Osaka University Medical School, Osaka, JAPAN. Joslin Diabetes Center Harvard Medical School, Boston, MA.
- 09:45 A14 **The Role of NDRG1 in Human Prostate Cancer**
Tina Napso, Fuad Fares
Department of Molecular Genetics, Carmel Medical Center and the Bruce Rappaport Faculty of Medicine, Technion-Israel Inst. of Technology, Haifa.
- 10:00 A15 **The Role of the Antioxidant Response Element Transcription System in the Enhancement of Vitamin D-Induced Differentiation of Leukemic Cells**
Irene Bobilev, Victoria Rossova, Joseph Levy, Yoav Sharoni, Michael Danilenko
Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University and Soroka Medical Center, Beer-Sheva

10:15 **Coffee Break and Poster Session**

POSTER SESSION (P48-P91)

THYROID/ ADRENAL/ BONE

- P48 **Penicillamine induced dysmorphogenesis and hypothyroidism in healthy infants born to a mother with Wilson disease, and in Wilson disease patients**
Aaron Hanukoglu, Batya Curiel, Drora Berkowitz, Arieh Levine, Mordehai Lorberboym
E. Wolfson Medical Center, Division of Pediatric Endocrinology, Division of Pediatric Gastroenterology, Institute of Nuclear Medicine, Tel-Aviv University Sackler School of Medicine. Rambam Medical Center, Division of Pediatric Gastroenterology
- P49 **Medullary thyroid carcinoma in Israel**
Anat Jaffe, Micha Barchana, Liana Tripto Shkolnik, Yoel Toledano, Michael Hopp.
Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera, Israel National Cancer Registry, Ministry of health, Jerusalem, Tel Aviv University; & HOP research, School of Public Health, Haifa University
- P50 **Complications of antithyroid agents: A summary: 1968-2007.**
Menachem Shapiro, Rosani Ness Abramov, Gavriela Solomen, Mark Nevin, Lima Witz (z"l), Louis Shenkman, Dan Nabriski
Meir Hospital Kfar Sava, Laniado Hospital Natanya

- P51 **Loss of thyrotropin set points in Down syndrome subjects as a possible mechanism for their hypothyroidism**
Joseph Meyerovitch, Michael Sherf, Felice Antebi, Zeev Hochberg
Health Planning and Policy Wing, Clalit Health Services, Tel Aviv. Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Affiliated to Tel Aviv University Sackler School of Medicine, Tel Aviv.
Department of Family Medicine, Ben Gurion University, Beer-Sheva.
- P52 **Neonatal hyperthyrotropinemia (HT): population characteristics, diagnosis, management and outcome after cessation of therapy**
Amnon Zung, Shiri Barkan, Yardena Tenenbaum-Rakover, Aaron Hanukoglu, Eli Hershkovitz, Tzvy Bistrizer, Orit Pinhas-Hamiel, Zvi Zadik
Pediatric Endocrinology, Kaplan Medical Center, Rehovot, Ha'Emek Medical Center, Afula, E. Wolfson Medical Center, Holon, Soroka Medical University, Beer-Sheva, Assaf-Harofe Medical Center, Tzrifin, Safra Children's Hospital, Sheba Medical Center, Tel-hashomer
- P53 **Hyponatremia in the elderly is associated with hypercortisolemia rather than adrenal insufficiency**
Tania Winchester, Moshe Sonnenblick, **Gabriel Munter**
Shaare Zedek Medical Center, Geriatrics and Internal Medicine Departments, Ben Gurion University
- P54 **5 α -reduced steroid metabolites interfere with steroidogenesis in the human adrenal tumor cells H295R**
Michal Lahav, Karina Tereshenkov, Zeev Blumenfeld, Carlos Knopf
Unit Of Endocrinology, OB/Gyn Dept., The Ruth And Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology; Laboratory of Metabolic Diseases and Steroid Metabolome, Department of Clinical Biochemistry, Rambam Medical Center, Haifa
- P55 **Bone Turnover after Alendronate Dose Reduction Following Prolonged Standard Full Dose Treatment in Postmenopausal Osteoporosis Patients**
Elena Segal, Zila Shen-Or, Batia Raz, Sophia Ish-Shalom
Metabolic Bone Diseases Unit, Endocrine Laboratory, Rambam Medical Care Campus, The Bruce Rappaport Faculty of Medicine, Technion -Israel Institute of Technology, Haifa
- P56 **Parathyroid Hormone Selective Venous Sampling (SVS): Surgical Adjunct to allow Minimally Invasive Parathyroidectomy in Sestamibi Scan Negative Patients**
Michael Krausz, Sophia Ish-Shalom, Elena Segal, Amos Ofer, Ahuva Engel, Michal Mekel
Department of Surgery A, Metabolic Bone Disease Unit, Invasive Radiology Unit, Rambam Medical Care Campus, Technion-Israel Institute of Technology, Haifa
- P57 **Plant-derived micronutrients inhibit estrogen signaling in mammary cancer but not in bone osteoblast-like cells**
Anna Veprik, Marina Khanin, Keren Hirsch, Michael Danilenko, Yoav Sharoni, Joseph Levy
Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University and Soroka Medical Center Beer-Sheva
- P58 **Immune cytokines modulate the levels of vitamin D receptor in keratinocytes**
Esther Ziv, Ruth Koren, Amiram Ravid
Felsenstein Medical Research Center, Department of Physiology and Pharmacology, Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University
- PUBERTY/GROWTH/GH/ IGF 1/ NEUROENDOCRINOLOGY**
- P59 **GnRH agonist treatment in girls with precocious puberty does not compromise post-pubertal uterine size**
Liat de Vries, Hadassa Goldberg-Stern, Moshe Phillip, Avi Ben-Haroush
Inst. for Endocrinology and Diabetes, Epilepsy Center, Schneider's Children Medical Center in Israel, Helen Schneider Hospital for Women, Rabin Medical Center, Beilinson Campus; Sackler school of Medicine, Tel-Aviv Univ.
- P60 **Nutrition-induced catch-up growth increases hypoxia inducible factor 1 α RNA levels**
Naomi Even Zohar, Jasmine Jacob, Ninnette Amariglio, Gideon Rechavi, Olga Potievsky, Moshe Phillip, Galia Gat-Yablonski

Inst. for Endocrinology and Diabetes, National Center for Juvenile Diabetes, Schneider Children's Medical Center of Israel, and Felsenstein Medical Research Center, Petach Tikva; Laboratory of Molecular Biology, Inst. of Hematology and Cancer Research Center, Sheba Medical Center, Tel-Hashomer

- P61 **Growth pattern and final height after cessation of gonadotropin-suppressive therapy in girls with central sexual precocity**
Liora Lazar, Anna Padoa, Moshe Phillip
Inst. for Endocrinology and Diabetes, Schneider Children's Medical Center of Israel, Petah Tikva, Dept. of Obstetrics and Gynecology, Assaf Harofe Medical Center, Zerifin, Sackler Faculty of Medicine, Tel Aviv University
- P62 **Glucagon stimulation test for childhood GH deficiency – timing of the peak is important**
David Strich, Nahum Terespolsky, David Gillis.
Pediatric Specialty Clinic, Endocrinology and Diabetes, CHS; Pediatric Endocrinology Clinic, Hadassah Univ. Hospital, Ein-Kerem, Jerusalem
- P63 **Relationship between antidepressants and the IGF-1 system in the brain: Possible role in cognition**
Michal Taler, Nurit Grunbaum-Novak, Hagit Cohen, Abraham Weizman, Irit Gil-Ad, Ronit Weizman
Laboratory of Biological Psychiatry, Felsenstein Medical Research Center, Campus Rabin, Petah-Tiqva and Tel-Aviv University; Mental Health Center, Ben-Gurion University, Beer-Sheva; Brull Community Mental Health Center, Tel-Aviv University
- P64 **Autocrine action of estrogen on the bovine mammary gland**
Yonatan Feuermann, Sameer Mabjeesh, Avi Shamay
Institute of Animal Science, Agricultural Research Organization, the Volcani Center, Bet Dagan; Department of Animal Science, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot
- P65 **Effects of selective somatostatin analogs and cortistatin on cell viability in cultured human non-functioning pituitary adenomas**
Hagit Padova, Hadara Rubinfeld, Moshe Hadani, Ilan Shimon
Institute of Endocrinology, Felsenstein Medical Research Center, Beilinson Hospital, Petah-Tiqva and Tel-Aviv University; Department of Neurosurgery, Sheba Medical Center, Tel-Hashomer
- DIABETES, OBESITY & ATHEROSCLEROSIS**
- P66 **Long-acting insulin analogues have mitogenic and antiapoptotic activities.**
Doron Weinstein, Zvi Laron, Haim Werner
Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Endocrine and Diabetes Research Unit, Schneider Children's Hospital, Petah Tikva
- P67 **The role of cytosolic isocitrate dehydrogenase in the regulation of insulin secretion and biosynthesis.**
Veronique Attali, Nurit Kaiser, Gil Leibowitz.
Endocrinology and Metabolism Service, Department of Internal Medicine, Hadassah-Hebrew University Medical Center, Jerusalem
- P68 **Interference of GLUT4 gene expression by siRNA impairs IGF-I regulated skeletal growth - A potential mechanism for type 1 diabetes associated growth defect**
Gila Maor, Roni Hazan Bril, Eddi Karnieli.
Anatomy and cell Biology, Faculty of Medicine, Technion; Endocrinology, Diabetes and Metabolism, Rambam Medical Center and Faculty of Medicine – Technion, Haifa
- P69 **Insulin-like mechanism of Glucose Tolerance Factor (GTF)**
Tal Mizrahi, Nitsa Mirsky. Haifa University
- P70 **Dynamics of Cell Cycle Machinery in Pancreatic Beta Cells**
Seth Salpeter, Tzvika Granot, Yuval Dor.
The Hebrew University-Hadassah Medical School Jerusalem
- P71 **Cystic fibrosis-related diabetes: the role of peripheral insulin resistance and beta-cell dysfunction**
Dalit Modan-Moses, Pinhas-Hamiel, Kineret Mazor, **Taipora Ziv**, Asher Barak, Yaakov Yahav, Ori Efrati
Pediatric Endocrinology Unit, Pediatric Pulmonology Unit, The Edmond and Lily Safra Children's Hospital, Tel-Hashomer
- P72 **Phosphodiesterase isoenzymes as target for enhanced insulin response in pancreatic beta-cells**

- Avital Dov**, Nasim Warwar, Eva Abramovitch, Rafael Neshner .
Endocrinology and Metabolism Service, Department of Medicine, Hadassah - The Hebrew University Medical Center, Jerusalem
- P73 **Characterization of Atherosclerotic Lesions in apoE-deficient mice with Scanning Electron Microscopy (SEM) of Wet Tissue**
Hofit Cohen , Aviv Shaish, Yehuda Kamari, Anya Vainshtein, Rafael Bizur, Arnon Afek, Shlomzion Shen, Dror Harats
The Institute of Lipid and Atherosclerosis Research, Sheba Medical Center, Tel-Hashomer and Sackler Faculty of Medicine Tel Aviv University; Quantomix Ltd., Rehovot, Israel
- P74 **Prevalence of dyslipidemia and other cardiovascular risk factors in obese children and adolescents referred to a tertiary care center in Israel**
Shlomit Shalitin, Moshe Phillip.
Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel, Petach Tikva, Sackler Faculty of Medicine, Tel Aviv
- P75 **Apparent Role of 3T3-L1 12-Lipoxygenase in preventing adipocyte apoptosis and facilitating fat accumulation**
Rona Limor, Anat Revzin, Gary Weisinger, Naftali Stern
Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center
- CANCER**
- P76 **Oncogenesis in mammary epithelial cells of transgenic mice expressing Stat5 variants: conditions for cancer development and characterization of gene expression profiles in tumors**
Tali Eilon, Itamar Barash.
The Volcani Center. Institute of Animal Science. Bet-Dagan
- P77 **Mechanisms for cancer prevention by dietary compounds: A role for the Electrophile/Antioxidant Response Element and the Nrf2 transcription factor**
Yaara Amosi, Karin Linnewiel, Keren Hirsch, Hagar Salman, Joseph Levy, Yoav Sharoni
Dept. of Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion Univ. of the Negev and Soroka Medical Center of Kupat Holim, Beer-Sheva.
- P78 **Affinity targeting of the cytotoxic drug daunomycin with 7-(o)-carboxymethyl daidzein improves the therapeutic response in animal model of human ovarian cancer**
Fortune Kohen, S. Katzburg , N. Nevo, R. P. Hodge, M. D. Renevey, V. Kalchenko, N. Stern, D. Somjen.
Biological Regulation, Veterinary Resources, Weizmann Institute of Science, Rehovot; Pharmacology and Toxicology, Texas University Medical Branch at Galveston, USA; Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center
- P79 **Hormonal regulation of hyaluronan synthase and its effect on the adhesion process of human epithelial ovarian carcinoma metastasis**
Yael Tzuman, Dorit Granot, Galit Mazoos, Nava Nevo, Michal Neeman.
Weizmann Institute of Science, Rehovot
- P80 **Synergistic antileukemic effects of vitamin D3 derivatives and rosemary polyphenols in a mouse acute myeloid leukemia model**
Ayelet Shabtay, Hagar Sharabani, Ze'ev Barvish, Joseph Levy, Yoav Sharoni, Michael Danilenko
Clinical biochemistry department, Ben Gurion university, Beer-Sheva
- SIGNALING**
- P81 **AHNAK - new mediator of Insulin action: Implications for GLUT4**
Dafna Ben-Yosef, Michal Armoni., Chava Harel, Eddy Karnieli
Technion Institute of Biotechnology, Rambam Medical Center
- P82 **Protein Kinase C Alpha And Protein Kinase C Delta Differentially Regulate Insulin Signaling in Hepatocytes.**
Tamar Brutman-Barazani, Shlomit Aga-Mizrachi, Miriam Horovitz-Fried, Chaya Brodie, Sanford Sampson.
Bar-Ilan University, Ramat-Gan
- P83 **Crosstalk between cAMP and vitamin D in keratinocytes**
Ms. Jennifer Romano, Amiram Ravid, Ruth Koren
Felsenstein Medical Research Center, Department of Physiology and Pharmacology, Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University

- P84 **Preparation and Characterization of Recombinant Leptin from Pufferfish, *Takifugu rubripes***
Michal Yacobovitz, Gili Solomon, Berta Levavi-Sivan, Arieh Gertler
Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem
- P85 **Insulin Increases Nuclear PKC Delta in L6 Skeletal Muscle Cells**
Miriam Horovitz-Fried, Tamar Brutman-Barazani, Sanford Sampson.
Faculty of Life Sciences, Bar-Ilan University
- REPRODUCTION**
- P86 **Novel roles for p38 and ERK1/2 in human ejaculated spermatozoa: regulation of flagellar motility, hyperactivation and acrosome reaction**
Tal Almog, Nir Etkovitz, Haim Breitbart, Zvi Naor
Dept. of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv Univ., The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan Univ.
- P87 **Are steroids obligatory mediators of LH/hCG triggered resumption of meiosis in mammals?**
Shmulik Motola, Malka Popliker, Alex Tsafiriri.
The Bernhard Zondek Hormone Research Laboratory, Department of Biological Regulation, the Weizmann institute of science, Rehovot
- P88 **Identification and Characterization of an Ovulation-Dependent Novel Gene**
Ketty Shkolnik, Shifra Ben-Dor, Dalia Galiani, Nava Nevo, Ariel Hourvitz, Nava Dekel
*Department of Biological Regulation, Department of Biological Services, Weizmann Institute, Rehovot
 IVF Unit, Department of Obstetrics and Gynecology, Chaim Sheba Medical Center, Tel-Hashomer*
- P89 **Induction of heparanase by lh during the ovulatory process**
Eyal Klipper, Ehud Tatz, Israel Vlodavsky, Uzi Moallem, Dieter Schams, David Wolfenson, Rina Meidan
Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot; Cancer and Vascular Biology Research Center, The Bruce Rappaport Faculty of Medicine, Technion, Haifa, Department of Dairy Cattle, Institute of Animal Sciences, Volcani Center, Bet-Dagan; Institute of Physiology, Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany
- P90 **Phospholipase D mediates hyperactivated motility in sperm capacitation.**
Sarit Bar-Sheshet Itach, Sara Rubinstein, Haim Breitbart
The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan Univ.
- P91 **FSH and hCG induced an increase in Lipoxygenase gene expression in both human granulosa-lutein cells and cumulus cells from women undergoing in vitro fertilization**
Shalom Bar-Ami, Reem Biadisy-Atamny, Adrian Ellenbogen Moshe Ben-Ami, Johnny S. Younis, Madeia Michaeli, Orit Radin, Anat Jaffe
Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera; Reproductive Medicine Unit, Department of Obstetrics and Gynecology, Poriya Medical Center, Tiberias; Institute of Evolution, Faculty of Science, University of Haifa, Haifa. Dept. of Obstetrics and Gynaecology, IVF Unit, Hillel Yaffe Medical Centre, Hadera

PL1 PLENARY LECTURE

Chair: **Z. Kraiem**

11:15 **Genetics of Thyroid Cancer Guides Design of Novel Therapeutic Strategies.**
Prof. James A. Fagin
Memorial Sloan-Kettering Cancer Center, New York, NY

12:10 **IES - BUSINESS MEETING**

12:45 **Lunch**

13:45 **Three parallel free communication sessions (FC)**

FC4 (A16-A20) ENDOCRINE PANCREAS, INSULIN SECRETION AND SIGNALING

Chair: **R. Neshet, A. Zung**

- 13:45 A16 **Incidences and age at disease onset of Type 1 Diabetes Mellitus among Israeli Ethiopians are correlated with the duration of exposure to a new environment**
Rafit Drori, Anat Jaffe
Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera; On behalf of the Israel IDDM Registry Study Group
- 14:00 A17 **Novel de novo heterozygous mutation in SUR1 presenting as hyperinsulinism (HI) in infancy followed by overt diabetes in adolescence – clinical and molecular studies**
IES PRIZE FOR BEST ABSTRACT IN CLINICAL RESEARCH
Maha Atwan, Jeremy Bushman, Show-Ling Shyng, Avital Perry, Sharona Tornovsky Babaey, Benjamin Glaser, David Zangen. *Hadassah Hebrew University Medical Center, Jerusalem, Oregon Health and Science University, Portland, USA*
- 14:15 A18 **PKC epsilon is involved in the control of insulin biosynthesis and secretion in pancreatic beta-cells**
Nasim Warwar, Esther Haber, Avital Dov, Eva Abramovitch, Erol Cerasi, Rafael Nesher
Endocrinology & Metabolism Service, Department of Medicine, Hadassah - Hebrew University Medical Center, Jerusalem
- 14:30 A19 **Rapamycin Treatment Exacerbates Nutrition-Induced Diabetes in Psammomys obesus**
Merav Fraenkel, Mali Ketzin Gilad, Yafa Ariay, Melis Karaca, Julien Castel, Christophe Magnan, Erol Cerasi, Nurit Kaiser, Gil Leibowitz.
Hadassah Medical Center, Hebrew Univ., Jerusalem Univ. Paris, Paris, France
- 14:45 A20 **The role of Thioredoxin interacting protein in β -cell dysfunction in Type 2 diabetes**
Maayan Shaked, Ety Bachar, Erol Cerasi, Nurit Kaiser, Gil Leibowitz
Endocrinology & Metabolism Service, Department of Medicine, Hadassah - Hebrew University Medical Center, Jerusalem

FC5 (A21-A25) ENDOCRINE CANCER

Chair: D. Gross, O. Cohen

- 13:45 A21 **Dual treatment of the prostate cancer cell line DU145 with the anti-EGFR antibody and 1,25-dihydroxyvitamin D3 efficiently suppresses cancer cell growth**
Vladimir Gavrilov, Olga Belochitski, Samuel Ariad, Vladimir Fridman, Shraga Shany.
Dept. of Clinical Biochemistry, Department of Oncology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev
- 14:00 A22 **Mechanism underlying the relationship between FSH, inhibin B (INB), anti-Mullerian hormone (AMH), and testosterone (T) secretion during long term treatment with Gonadotropin releasing-hormone agonist (GnRHa) in prostate cancer**
Talia Eldar-Geva, Gad Liberti, Boris Chertin, Alon Fridman, Hadassa Hartman, Amicur Farkas, Irving Spitz
Shaare-Zedek Medical Center; Institute of Hormone Research, Jerusalem
- 14:15 A23 **Registry of Adrenocortical Carcinoma in Israel - A preliminary report on 104 patients**
Anat Jaffe, Micha Barchana, Liana Tripto Shkolnik, I Liphshitz, Yoel toledano, Michael Hopp, Carlos Benbassat
Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera; Israel National Cancer Registry, Ministry of health, Jerusalem; Tel Aviv University; & HOP research: School of public Health, Haifa University; Institute of Endocrinology & Metabolism Beilinson Hospital, Petah-Tiqva
- 14:30 A24 **Hospital-based registry of differentiated thyroid cancer: a useful resource to improve the clinical management of thyroid cancer patients.**
Carlos Benbassat, Dania Hirsch, Gloria Zvetov, Ilana Shraga-Zlutzky, Ruth Weinstein, Monica Gaspar, Hanna Bernstine, Adam Steinmetz, Ronit Kalmanovitch, Joelle Singer, Ilan Shimon
Endocrine Inst., Dept of Nuclear Medicine, Beilinson Hospital, Petah-Tiqva
- 14:45 A25 **Differentiated thyroid carcinoma in the pediatric population – comparison between prepubertal children and adolescents**
Yael Lebenthal, Moshe Phillip, Liora Lazar.
Institute for Endocrinology and Diabetes, Schneider Children's Medical Center of Israel, Petah Tikva

FC6 (A26-A30) ENDOCRINOLOGY OF REPRODUCTION

Chair: P. Kraicer, I. Koch

- 13:45 A26 **The Orientation of The Alpha and Beta subunit domains in single-chain bovine LH analogs affects the secretion and steroidogenic response in vitro**
Moran Grinberg, Sigal Nakav, Svetlana Pen, Ada Dantes, Ruth Braw-Tal, Abraham Amsterdam, David Ben-Menahem.
Department of Clinical Pharmacology, The Faculty of Health Sciences, Ben-Gurion Univ. of the Negev, Beer-Sheva; Inst. of Animal Science, Agricultural Research Organization, The Volcani Center, Beit Dagan; Dept. of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot
- 14:00 A27 **LH-induced caspase activation in rat preovulatory follicles is coupled to mitochondrial steroidogenesis**
Keren Yacobi, Alex Tsafirri, **Atan Gross**.
Dept. of Biological Regulation, The Weizmann Institute of Science, Rehovot
- 14:15 A28 **Endometrial-trophoblast interaction: The role of Plexin-B1 and Progesterone receptors.**
Haggar Harduf, Shlomit Goldman, Eliezer Shalev.
Rapaport faculty of Medicine, Technion, Haifa. Laboratory for research in reproductive science, Dept. of Obstetrics and Gynecology, Ha'Emek Medical Center, Afula
- 14:30 A29 **The role of Xenopus membrane progesterone receptor beta in mediating the effect of progesterone on oocyte maturation.**
Liat Josefsberg Ben-Yehoshua, Andrea L Lewellyn, Peter Thomas, James L. Maller.
Howard Hughes Medical Institute, Univ. of Colorado Health Sciences Center, Denver; Marine Sciences Institute, Univ. of Texas, Port Aransas
- 14:45 A30 **Elevated ET-2 in the young bovine corpus luteum: induction by LH and hypoxia**
Eyal Klipper, Yonit Mastich, Dieter Schams, Rina Meidan
Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot; Institute of Physiology Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany

15:00 **Coffee Break**

SL Two Parallel Symposia

SL1 Israeli Endocrine Society Guidelines for Thyroid Cancer

Chair: Y. Liel, J. Arbelle

- 15:15 S1 **Israeli Guidelines for Thyroid Cancer –**

Dr. Jonathan Arbelle
Maccabi Sherutey Briut, Beer-Sheva

- 16:00 S2 **Case presentations –**

Dr. Carlos Benbassat
Rabin Medical Center, Petah-Tiqva

- 16:20 S3 **Open Discussion**

SL2 Comparative Endocrinology

Chair: A. Shamay, M. Friedman-Einat

- 15:15 S5 **The Effect of Leptin and GnRh on the Reproduction of Tilapia as a Model Fish**

Dr. Bertha Sivan
Department of Animal Science, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot

- 15:40 S6 **The Role of Retina and Extra Retinal Photo Stimulation in Reproductive Activities of Domestic Birds**

Dr. Israel Rosenboim
Department of Animal Science, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot

- 16:05 S7 **Multi-Level Regulation of Milk Protein Synthesis**

Dr. Itamar Barash
Institute of Animal Science, Agricultural Research Organization, the Volcani Center, Bet-Dagan

- 16:30 S8 **Prolactin, Leptin and The Mammary Gland**

Dr. Avi Shamay
Institute of Animal Science, Agricultural Research Organization, the Volcani Center, Bet-Dagan

Wednesday, April 18, 2007

- 07:45 **Registration (for those not registered on the 1st day) and poster hanging**
- 08:30 **Three parallel free communication sessions (FC)**
- FC7 (A31-A36) BONE METABOLISM AND VITAMIN D**
Chair: A. Ravid, E. Segal
- 08:30 A31 **Vitamin D attenuates the keratinocyte inflammatory response to TNF**
Mor Miodovnik, Amiram Ravid, Ruth Koren
Felsenstein Medical Research Center, Department of Physiology and Pharmacology, Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University
- 08:45 A32 **MS-KIF18A kinesin is regulated by estrogen and binds estrogen receptor**
Margalit Zusev, Dafna Benayahu.
Department of Cellular and Developmental Biology, Sackler school of Medicine, Tel-Aviv University
- 9:00 A33 **Effects of androgens and estrogens on 12- and 15-Lipoxygenase Expression and Activity in Cultured Human Female Derived Osteoblasts.**
Dalia Somjen, Sara Katzburg, Esther Knoll, Orly Sharon, Rona Limor, David Hendel, Naftali Stern
Inst. of Endocrinology, Metabolism and Hypertension, Tel-Aviv Sourasky Medical Center, and The Sackler Faculty of Medicine, Tel-Aviv Univ.; Dept. of Orthopedic Surgery, Sharei- Zedek Medical Center, Jerusalem
- 09:15 A34 **Clinical characteristics of patients treated with Teriparatide after long term antiresorptives treatment in a single tertiary referral metabolic clinic**
Gloria Tsvetov, Carlos Benbassat, Ilana Shraga-Slutzky, Varda Eshed
Endocrine Institute, Rabin Medical Center, Petah Tikva, Sackler Faculty of Medicine, Tel Aviv University; Medical Department, Eli Lilly, Israel
- 09:30 A35 **The involvement of Estrogen in accelerated Osteogenesis in skeletal growth center: A proposed mechanism**
Marina Gurman, Moshe Phillip, **Gila Maor**.
Department of Anatomy and Cell Biology, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa; Institute of Endocrinology and Diabetes, Schneider Children's Medical Center, Petach Tikva
- 09:45 A36 **Vitamin D Deficiency in Oncologic Patients - an Ignored and Potentially Life Threatening Condition, the Role of Osteoblastic Activity in Hypocalcemia**
Sophia Ish-Shalom, Shira Felder, Elena Segal, Hedva Yoffe- Sheinman, Mira Volner, Eliahu Gez, Batia Raz, Zila Shen- Or Nissim Haim.
Metabolic Bone Diseases Unit, Department of Oncology, Endocrine Laboratory, Ramabam Health Care Campus, Haifa; The Bruce Rappaport Faculty of Medicine, Technion -Israel Institute of Technology, Haifa
- FC8 (A37-A41) ADRENAL**
Chair: N. Stern, A. Hanukoglu
- 08:45 A37 **Evaluation of the Hypothalamic-Pituitary-Adrenal Axis and Metabolic Profile of Women with Polycystic Ovary Syndrome**
Elvira Chen, Caroline Apovian, Suzy Kovatz, Ghazy Ganem, Pnina Rotman-Piekelnny, Michael Pomeranz, Eliahu Weiss Menachem Shapiro, Louis Shenkman, **Rosane Ness-Abramof**
Departements of Medicine B, C, E, Department of Obstetric and Gynecology, Endocrine Unit & Endocrine Laboratory, Sapir Medical Center, Kfar Saba; Kupat Holim Dgani, Hadara; Section of Endocrinology, Diabetes, and Nutrition, Boston Medical Center, Boston
- 09:00 A38 **Disease control and sleep/activity quality by morning or evening replacement therapy in congenital adrenal hyperplasia**
Alina German, Suhir Suraiya, Yardena Tenenbaum-Rakover, Ilana Koren, Giora Pillar, Ze'ev Hochberg.
Clalit HMO, Haifa; Pediatric Endocrinology Unit, Meyer Children's Hospital, and Sleep Laboratory, Rambam Medical Center Haifa; Pediatric Endocrine Unit, Ha'Emek Medical center, Afula; Technion Faculty of Medicine, Haifa

- 09:15 A39 **Mutations in epithelial sodium channel (ENaC) beta subunit gene responsible for multi-system pseudohypoaldosteronism in ashkenazi families**
Oded Edelheit, Israel Hanukoglu, Matanel Tefilin, David Gillis, David H. Zangen, Gheona Altarescu, Aaron Hanukoglu
Dept. of Molecular Biology, College of Judea and Samaria, Ariel; Tel-Aviv Univ., Sackler Medical School; Dept. of Pediatrics, Hadassah-Hebrew Univ. Medical Center, Jerusalem; Genetics Unit, Shaare Zedek Medical Center; Div. of Pediatric Endocrinology, E. Wolfson Hospital, Holon
- 09:30 A40 **Absence of the PPARalpha (PPARa) gene abolishes hypertension, and attenuates atherosclerosis through downregulation of the Renin-Angiotensin-Aldosterone system in the Tsukuba hypertensive mouse (THM)**
Karen M. Tordjman, Clay F. Semenkovich, Trey Coleman, Etty Osher, Michal Vechoropoulos, Nafatli Stern
Inst. of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center; Div. of Endocrinology, Metabolism & Lipid Research, Washington Univ. School of Medicine, St. Louis, Missouri, USA
- 09:45 A41 **Inhibition of 12-lipoxygenase by baicalein reduced in vitro cell proliferation and induced apoptosis in two human primary adrenocortical carcinoma**
Anat Jaffe, Sholamit Karby, Keren Cohen, Shalom Bar-Ami.
Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera
- FC9 (A42-A47) INTRACELLULAR SIGNALING MECHANISMS**
Chair: F. Fares, Z. Naor
- 08:30 A42 **Role of PKC in GnRH activation of Extracellular Signal-Regulated Kinase (ERK) and Jun N-Terminal Kinase (JNK).**
Masha Dobkin-Bekman, Rony Seger, Zvi Naor
Department of Biochemistry, Tel Aviv Univ., Ramat Aviv; Department of Biological Regulation, The Weizmann Institute of Science Rehovot
- 08:45 A43 **Placental Expression of PKBalpha/Akt1 Improves Survival but does not Rescue PKBalpha/Akt1- deficient Embryos from Intrauterine Growth Retardation**
Vicki Plaks, Elina Berkovitz, Tamara Berkutzki, Rebecca Haffner, Alon Harmelin, Brian A. Hemmings, Nava Dekel Michal Neeman.
Biological Regulation, Veterinary Resources, The Weizmann Institute of Science, Rehovot, Israel; Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland,
- 09:00 A44 **Activin as a Possible Anti-Apoptotic Agent: Relevance for Neurodegenerative Diseases.**
Lana Kupersmidt, Kupersmidt L, Amit T, Orith Bar-Am, Youdim M.B.H, Blumenfeld Z
The Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa
- 09:15 A45 **Vitamin D induces COX-2 in human keratinocytes**
Efrat Buchner, Ruth Koren, Amiram Ravid.
Felsenstein Medical Research Center, Department of Physiology and Pharmacology, Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University
- 09:30 A46 **Engineered Thyrotropin Variants with Increased In vivo Activity and High Serum Stability**
Nael Azzam, Fuad Fares.
Department of Molecular Genetics, Carmel Medical Center and Faculty of Science, University of Haifa
- 9:45 A47 **Administration of leptin antagonist attenuates Concavalin A- and Pseudomonas Exotoxin A-induced experimental hepatitis and thioacetamide-induced hepatic fibrosis**
Arieh Gertler, Gila Solomon, Muhammed Ali, Rafi Bruck, Zamir Halpern, Eran Elinav.
Inst. of Biochemistry, Food Science & Nutrition, The Hebrew Univ. of Jerusalem; Gastroenterology & Liver Inst., Tel Aviv Sourasky Medical Cntr

- 10:00 **Coffee Break and Poster session**
- SL3 Endocannabinoid System (Sponsored by Sanofi-Aventis)**
Chair: R. Dresner-Pollak
- 10:45 **The Endocannabinoid System: an Overview of its Physiological Roles**
Prof. Raphael Mechoulam
The Hebrew University of Jerusalem
- 11:20 **The Role of the Endocannabinoid System in Common Obesity**
Dr. Gabriella Lieberman
Sheba Medical Center, Tel-Hashomer
- 11:45 **Endocannabinoid Regulation of Bone Remodeling**
Prof. Itai Bab
The Hebrew University of Jerusalem
- PRIZE LECTURES AND PRIZE ANNOUNCEMENTS**
Chair: B. Glaser
- 12:15 **Recognition Award for Special Contribution to Endocrinology in Israel – to Prof. Zvi. Laron**
- 12:25 **LINDNER PRIZE – In vitro Models of Human Pituitary Hormone Regulation**
Dr. Ilan Shimon
Rabin Medical Center, Petah Tiqva
- 12:55 **CHOWERS PRIZE – A Servant to two Masters: Tissue-Specific Regulation of Steroidogenic P450 (Cyp11a) in the Ephemeral Placenta and Cycling Ovary**
Noa Sher
The Hebrew University of Jerusalem
- Low Expression of COX-2, Reduced Cumulus Expansion, and Impaired Ovulation in SULT1E1-deficient mice**
Dr. Eran Gershon
Biological Regulation, Weizmann Inst., Rehovot
- 13:25 **Announcement of Best Clinical and Basic Abstracts; Best Posters**
- 13:40 **Lunch**
- SL4 Endocrine-related Cancer (Sponsored by Novartis)**
Chair: H. Werner
- 14:45 S9 **Prostate Cancer: A Disease of Ectopic Steroidogenesis Regulated by Insulin-like Growth Factors**
Prof. Steve Plymate
Univ. of Washington, Seattle, WA
- 15:15 S10 **Breast Cancer: Endocrine and Genetic Risk Factors - an Update**
Prof. Eitan Friedman
Sheba Medical Center, Tel-Hashomer
- 15:45 S11 **Obesity and Cancer: Novel Molecular Mediators**
Dr. Hana Kanety
Sheba Medical Center, Tel-Hashomer
- 16:15 S12 **Dietary Intervention in Steroid Hormone Action**
Prof. Yoav Sharoni
Ben-Gurion Univ. of the Negev, Beer-Sheva
- 17:00 **Closure**

Role of p38-MAPK and ErbB receptors pathways in the induction of IRS-1 serine phosphorylation and insulin resistance by TNF

Miss Yafit Rachminov¹ Mr. Ehud, Ehud Barhurdar¹ Prof. Uri Nir¹ Mrs. Maayan Barnea³ Prof. Zecharia Madar Madar³ Prof. Avi, Avi Karasik² Dr. Rina Hemi² Dr. Hannah Kanety²

¹*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University*

²*Institute of Endocrinology, Sheba Medical Center*

³*Faculty of Agriculture, The Hebrew University of Jerusalem*

Introduction: IRS-1 serine (Ser) phosphorylation has a key role in obesity-induced insulin resistance and is associated with the increased levels in obesity of the proinflammatory cytokine TNF and ROS. However, the mechanisms that link these cellular stress factors to IRS-1 phosphorylation and impaired insulin action are not fully understood. Previously, we demonstrated that exposure of rat hepatoma Fao cells to TNF leads to p38MAPK (p38) activation, in a redox-dependent manner, which mediates the transactivation of ErbB2/3 receptors. This in turn triggers a PI3K cascade, which induces IRS proteins Ser phosphorylation, resulting in insulin signaling desensitization. Here, we further examined the general relevance of p38-linked ErbB receptors transactivation in inflammatory and stress-induced liver insulin resistance.

Patients / Methods: The interplay between stress stimuli, p38 stress kinase, ErbB receptors transactivation and IRS-1 phosphorylation has been examined in vitro in cultured human hepatoma cells HepG2, following their exposure to TNF, and in vivo in high fat diet (HFD)-induced obese mice, as well as in lean mice subsequent to the manipulation of their liver p38 expression by injection of an p38WT adenovirus.

Results: TNF induced IRS-1 phosphorylation in HepG2 cells on Ser312, that is located proximal to the PTB domain, and Ser616/Ser636 that are adjacent to Tyr612, a PI3K binding site. This phosphorylation impaired insulin action, as demonstrated by diminished insulin-induced Tyr612 phosphorylation, and reduced Akt phosphorylation. In parallel, TNF activated p38 and stimulated the Tyr phosphorylation of ErbB1, the major ErbB receptor in HepG2 cells. Overexpression of WT-p38 augmented TNF effects on ErbB1 and IRS-1 phosphorylation, while inhibition of p38 by its specific inhibitor SB203580, or by overexpression of a DN-p38 suppressed TNF-induced ErbB1 transactivation, reduced its stimulatory effect on IRS-1 Ser phosphorylation, and correspondingly attenuated its inhibitory effect on insulin-dependent IRS-1 Tyr612 phosphorylation. In addition, specific inhibitors of the ErbB1 tyrosine kinase (PD168393 and AG537) prevented TNF-induced IRS-1 Ser phosphorylation, but not p38 activation, supporting the notion that p38 is an upstream mediator of TNF-induced ErbB1 transactivation. The role of p38 and ErbBs in insulin resistance induction was further supported by in vivo experiments; Liver p38 activity and ErbB1/3 expression were increased in C57Bl/6 mice fed a HFD, while insulin-induced IRS-1 Tyr612 phosphorylation was impaired in lean C57Bl/6 mice injected with the p38WT adenovirus, concomitant with increased ErbB1 Tyr phosphorylation.

Conclusions: Our findings identify p38 as a cytokine- and oxidative stress-induced protein kinase responsible for ErbB receptors transactivation, and suggest that ErbB signaling networks are playing a pivotal role in TNF-induced insulin resistance. This paradigm points to potential sites for therapeutic intervention using either antioxidants or kinase inhibitors to remedy insulin-resistance.

Abstract Code: A2

Cytosolic Protein Tyrosine Phosphatase epsilon (cytPTPe) is a Negative Regulator of Insulin Signaling in Skeletal Muscle.

Dr. Shlomit Aga-Mizrachi¹ Dr. Avraham I., Avraham I. Jacob¹ Ms. Tamar Brutman-Barazani¹ Mrs. Asia Bak¹ Prof. Ari Elson² Prof. Sanford R., Sanford R. Sampson¹

¹*Bar-Ilan University, Ramat-Gan*

²*Weizmann Institute of Science, Rehovot*

Introduction: A major characteristic of type II (non-insulin dependent) diabetes is insulin resistance in peripheral tissues including liver, fat and skeletal muscle. The molecular mechanism underlying insulin resistance is not yet clear. It is believed, however, to involve the impairment of insulin receptor (IR) signal transduction. While positive regulatory events triggered by insulin binding to IR have been well-documented, the mechanism by which the activated IR is returned to the basal status through protein tyrosine phosphatases (PTPs) is not completely understood. Recently, studies have focused on the involvement of PTPs and how they might influence IR signaling. In this study, we examined the possibility that cytPTPe is involved in regulation of IR tyrosine phosphorylation and IR signaling in L6 skeletal muscle cells.

Patients / Methods: Studies were performed on L6 skeletal muscle cells. cytPTPe was over expressed by using pBABE retroviral expression vectors. In addition, we inhibited cytPTPe by RNA silencing. Tyrosine phosphorylation of insulin signaling proteins was determined by Western blotting utilizing phosphospecific antibodies.

Results: We found that insulin induced both rapid translocation of cytPTPe to the cell membrane fraction and association with IR. Interestingly, this association occurred in the plasma membrane, and on stimulation with insulin the 2 proteins internalized together. Moreover, insulin stimulation increased the association of the 2 proteins, and it appeared that almost all internalized IR was associated with cytPTPe. In studies on insulin-induced tyrosine phosphorylation of IR, we found that knockdown of cytPTPe by expression of RNAi increased the effect of insulin. Moreover, overexpression of WT cytPTPe reduced insulin-induced tyrosine phosphorylation of IR. Similarly, tyrosine phosphorylation of IRS-1, as well as phosphorylation of PKB and GSK, and insulin-induced stimulation of glucose uptake, were increased in preparations in which PTPe was knocked down and decreased in preparations in which PTPe was over expressed.

Conclusions: We conclude that cytPTPe is a negative regulator of IR signaling in skeletal muscle.

Crosstalk between inflammation and insulin resistance in insulin target tissues: NFkB represses GLUT4 gene promoter

Mrs. Shiri Karni¹ Dr. Michal, Michal, Michal, Michal Armoni² Dr. Chava Harel
¹ Dr. Simcha Milo ² Prof. Eddy Karnieli^{1,2}

¹*Techion- Israel Institute of Technology*

²*Rambam Medical Center*

Introduction: The link between inflammation, insulin resistance and type 2 diabetes mellitus (DM2) is now well-established. Since NFkB activity is enhanced in DM2, we studied its role in regulating glucose transporter 4 (GLUT4) gene in bona fide insulin target tissues.

Patients / Methods: Endogenous gene expression was studied by QRT-PCR in cardiac muscle biopsies obtained during elective coronary surgery from 6 DM2 and 8 normoglycemic patients (%HbA1c 7.9,±0.7 vs. 5.7,±0.3 p<0.05), matched for sex, age and BMI.

Results: We found that DM2 was associated with 20% increased and 20% decreased levels of p65 and GLUT4 mRNAs, respectively, while p50 levels remained unchanged.

GLUT4 regulation at transcriptional level was studied in vitro in primary rat adipocytes (PRA) using co-transfection assays. Transient transfection of 0-5ug of either p65 or p50 subunits of NFkB, dose-dependently suppressed activity of the co-expressed GLUT4 promoter (GLUT4-P), to 40% and 20% of basal levels, respectively. Co-transfection with equimolar amounts of both p65 and p50 had an additive 50% repression effect of GLUT4-P activity. 5Yf-deletion analysis of GLUT4-P revealed that two distinct promoter regions mediate the effects of p50 and p65. These regions contain several NFkB consensus motifs that may serve as potential binding sites for each subunit.

Conclusions: We thus suggest that NFkB subunits repress GLUT4 gene transcription in insulin target tissues, and that these effects are mediated via specific sites on the GLUT4 promoter. Elevated p65 mRNA levels observed in human cardiac muscle may impair GLUT4 expression in this tissue, contributing to diabetic cardiomyopathy observed in DM2 patients. Thus, beyond the previously-described crosstalk between inflammatory and insulin signaling pathways, we now show a novel intersection point at the level of NFkB trans-repression of the GLUT4 gene promoter. Hence, NFkB is introduced as potential therapeutic target for DM2 and insulin resistance.

Identification and Characterization of Novel Adiponectin Receptor 1 Isoforms

Dr. Rina Hemi¹ Miss Reut, Reut Ashwall² Mrs. Reut Rosenblum² Prof. Avi Karasik¹ Dr. Hannah Kanety¹

¹*Institute of Endocrinology, Sheba Medical Center*

²*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University*

Introduction: Adiponectin is an adipocyte-derived abundant plasma protein, with insulin-sensitizing, anti-inflammatory and anti-atherogenic properties. Its levels are reduced in obese and insulin-resistant subjects, as well as in patients with diabetes and cardiovascular disease. Two distinct adiponectin receptors were cloned; AdipoR1 and AdipoR2. Reduced expression or impaired function of adiponectin receptors by genetic or environmental factors may lead to adiponectin resistance and thereby may accelerate the development of diabetes and cardiovascular diseases. Alternative mRNA splicing is an important mechanism for the generation of structural and functional diversity in proteins under physiological and pathophysiological conditions. The aim of the current study was to identify and characterize novel alternatively spliced variants of human AdipoR1 receptors.

Patients / Methods: By employing bioinformatics, molecular biology and biochemical techniques, we identified novel alternatively spliced variants of AdipoR1, evaluated their relative mRNA and protein expression in various adult and fetal human tissues, and assessed their impact on adiponectin signaling.

Results: By using LEADS Human Transcriptome Database (Compugen), 8 predicted mRNA transcripts (T1-T8) of AdipoR1 that encode 4 predicted protein products were identified. Using specific primers, designed according to this data, the existence of T1, T2, T3, T5 and T8 transcripts in various human tissues was established by RT-PCR and their identity was confirmed by DNA sequencing of their PCR products. Quantitative real-time PCR analysis revealed distinct expression of the different AdipoR1 transcripts in adult and fetal liver, muscle, adipose and brain tissues, indicating that they are subjected to tissue as well as to developmental regulation; T1, that encodes for the WT protein is abundant in adult liver and adipose tissues, whereas T3 which has an altered 5' UTR and also encodes the WT protein, is abundant predominantly in adult skeletal muscle. However, in fetal skeletal muscle as in the fetal liver, T1 is the dominant transcript. T5, another newly identified transcript, which encodes a truncated isoform, with a distinct sequence at the C-terminus, is significantly expressed in adult liver and pituitary. Western blot analysis with commercially available antibodies against WT-AdipoR1 and specific custom made antibodies against the truncated isoform of AdipoR1 (Tru-AdipoR1) demonstrated that the protein expression of AdipoR1 isoforms in human tissues is consistent with the real time PCR results; the WT-AdipoR1 is the major receptor in the muscle whereas in liver and pituitary, there is a significant expression of Tru-AdipoR1 in addition to the WT isoform. By overexpression in HEK293 cells preliminary biochemical and functional characterization of both isoforms and their impact on adiponectin signaling was obtained.

Conclusions: In the current study we identified novel alternatively spliced variants of human AdipoR1 receptors that are tissue and developmentally regulated. Understanding their regulation and the interplay between these isoforms may enable the development of new strategies to enhance adiponectin signal and thereby its anti-diabetic and anti-atherogenic effects.

Regulation of the gene encoding GPR40, a potential missing link between obesity and diabetes

Gabriela Ridner¹ Reut, Reut Bartoov-Shifman¹ Keren Bahar¹ Michael D. Walker¹

¹*Weizmann Institute of Science-Rehovot*

Introduction: GPR40 is a member of a small family of genes clustered on mouse chromosome 7, that encode receptors for fatty acids. GPR40 is expressed preferentially in pancreatic beta cells and appears to play a key role in mediating both physiological and pathological effects of fatty acids on beta cells.

Patients / Methods: We have used reporter gene assay, 5' RACE, DNase I and MNase sensitivity assay, chromatin immunoprecipitation and gel mobility assay to characterize GPR40 gene transcription.

Results: We have identified both the GPR40 transcription start site (TSS) and core promoter. Evolutionary comparisons of the GPR40 locus revealed conserved regions within the GPR40 5' flanking sequence, which we designated HR2 and HR3. The GPR40 locus has a more open conformation in beta compared to non-beta cells as indicated by the presence of beta cell-specific DNase I hypersensitive sites in the HR2 and HR3 regions, and distinct nucleosomal positioning on the HR2 region in beta cells. Consistent with this, chromatin from beta cells, but not other cells, was enriched for acetylated histone H3 in HR2 and HR3. Deletion of the HR2 sequence, led to a 90% reduction in GPR40 promoter activity, indicating the existence of a critical transcriptional control element within HR2. Systematic mutagenesis of HR2 revealed the importance of two elements that reduced promoter activity dramatically. The first element contains potential binding sites for homeodomain transcription factors: its elimination reduced transcriptional activity by ~80%. In vitro DNA binding experiments showed that this element can specifically bind PDX1, a crucial beta cell-specific transcription factor of the homeodomain family. The in vivo relevance of this binding was tested by quantitative chromatin immunoprecipitation experiments that showed PDX1 occupancy on the HR2 region in beta cells. The second element contains an E box sequence which is recognized by transcription factors from the bHLH family and its elimination reduced transcriptional activity by ~50%. We have shown that BETA2, a beta cell-specific transcription factor is able to bind the HR2 region both in vitro and in vivo.

Conclusions: Taken together, our results demonstrate that cell-specific expression of the GPR40 gene is controlled at the transcriptional level through HR2, a potent beta cell-specific enhancer, and involves a characteristic chromatin organization of the locus. Understanding the global mechanisms controlling regulation of the GPR40 locus is of major importance since defective regulation of GPR40 gene transcription might lead to inappropriate responses of beta cells to nutrients and perturbed glucose homeostasis

Evidence that prenatal maternal serotonin reuptake inhibitors (SSRIs) therapy induces changes in anthropometrical parameters and in the hormonal profile of newborns

Dr. Diana Prokonov¹ Dr. Irit, Irit, Irit, Irit Gil-Ad^{2,3} Dr. Rachel Maayan² Mr. Amichai Zolokov^{2,3} Prof. Abraham Weizman^{2,3} Dr. Shmuel, Shmuel, Shmuel, Shmuel Davidson^{1,3}

¹*Department of Neonatology Rabin Medical Center*

²*Lab Biological Psychiatry, Felsenstein Medical research Center*

³*Tel-Aviv University*

Introduction: Brain neurotransmitters e.g serotonin (5HT) and norepinephrine (NE) regulate pituitary hormones secretion. Antidepressants like serotonin reuptake inhibitors (SSRIs) alter brain 5HT and NE. SSRIs are widely used during pregnancy. The data on the somatic growth of infants exposed to SSRIs in utero, as well as the data on the Hypothalamic-Pituitary-Adrenal axis is limited and controversial.

The aim of our study was to determine the anthropometrical parameters and the hormonal profile in cord blood of infants exposed to maternal SSRIs during pregnancy.

Patients / Methods: The study was conducted in Rabin Medical Center. 22 infants of mothers using SSRIs during pregnancy (whole period) and 20 control infants were included. Cord blood level of Cortisol, IGF-1, Prolactin, TSH, DHEA, DHEAS and urine 5HIAA (Serotonin metabolite in the urine) were determined using RIA or ELISA methods. IGF-1 receptors in the placenta was evaluated by western blot. Neonatal withdrawal symptoms were determined in the SSRIs group using the Finnegan score.

Results: We found in male neonates impaired birth weight, length and head circumference. Serum IGF-1 and DHEAS levels were significantly lower than in the control male group. In female neonates, body length and cortisol levels were lower than in controls. In both sexes SSRIs exposed neonates plasma TSH levels were significantly higher than in controls, and a total of 27% of the newborn showed borderline high levels. In the SSRIs infants, the Finnegan score was positively correlated, though not significantly, with cord blood Cortisol ($r=0.4$, $p=0.09$). Birth weight was positively correlated with cord IGF-1 ($r=0.6$, $P<0.005$) and negatively correlated with urine 5HIAA ($r=-0.52$, $p<0.02$). Cord IGF-1 and urine 5HIAA were negatively correlated ($r=-0.42$, $p=0.06$). IGF-1 receptors expression in the placenta was higher in the SSRIs treated group.

Conclusions: Infants exposed to SSRIs in utero show impaired intrauterine growth, a tendency toward decreased IGF-1, and high frequency of borderline high TSH levels. IGF-1 receptors expression probably increases as a compensatory mechanism. It seems that follow up of these children is important.

IGF-I Replacement Therapy in Children with Congenital IGF-I Deficiency Restore Heart Dimension and Function in Children with Laron Syndrome

Prof. Zvi Laron¹ Dr. Olga, Olga Kisselgoff² Prof. David M. Steinberg³ Dr. Yzhar Toren³ Dr. Miche Feinberg⁴ Dr. Mickey, Mickey Scheinowitz⁵

¹*Endocrinology and Diabetes Research Uni, Schneider Children`s Medical Cente, Petah-Tikva*

²*Cardiac Clinic, Schneider Children`s Medical Cente, Petah-Tikva*

³*Faculty of Exact Sciences, Tel-Aviv University*

⁴*The Heart Institute, Sheba Medical center*

⁵*Neufeld Cardiac Research Institute, Tel-Aviv University*

Introduction: Untreated patients with congenital growth hormone deficiency (GHD) and IGF-I deficiency are characterized not only by dwarfism but also by acromicria and organomicria, such including the heart. Aim of Study: To assess cardiac dimension and function in very young patients with Laron syndrome (LS) undergoing IGF-I replacement therapy, 120-150mcg/Kg once daily (Fugisawa, Japan).

Patients / Methods: Two to seven echocardiographic measurements were performed during therapy of male (n=4) and female (n=4) LS pts., mean (SD) age: 7.1 (3.6) yrs (range 1.6-11.6 yrs) weight: 16.1 (9.7) kg and height of 89.9 (18.5) cm. As aged- and gender-matched controls served 44 healthy children, age: 8.7 (5.5) yrs, weight: 36.1 (22.4) kg, and height: 129.7 (33.1) cm. Data of LS patients were normalized to body surface area and compared to the control group as well as nomograms of echocardiographic parameters for this age group (Silverman, 1993).

Results: Left ventricular diastolic and systolic dimensions (LVDD/ LVSD, mm) and LV mass (gr) were significantly smaller in boys and girls with LS compared with controls, while the shortening fraction (%) and intraventricular septum thickness (mm) were similar; 35 (5) % vs. 38 (4) % and 0.63 (0.18) vs. 0.66 (0.16) mm, respectively. When compared with standard values for this age group, all treated LS patients were within 1 standard deviation of the mean.

Conclusions: IGF-I therapy of young patients with IGF-I deficiency restores LV dimension and function in children with cong. IGF-I deficiency.

11 β HSD-1 activity in the programming of growth retardation and metabolic morbidity in SGA

Dr. Nehama Zuckerman-Levin¹ Dr. Larisa, Larisa Ziblin¹ Dr. Carlos Knopf²
Mrs. Oshrat Baruch² Prof. Ze'ev Hochberg^{1,3}

1Pediatric Endocrinology, Meyer Children's Hospital, Rambam Medical Center

2Clinical Biochemistry, Rambam Medical Center

3 Faculty of Medicine, Technion – Israel Institute of Technology

Introduction: SGA children are programmed from early on to develop the metabolic syndrome. A subgroup do not catch up, remain short, and may benefit from GH therapy. 11 β HSD-1 is expressed mainly in visceral fat, and implicated in metabolic morbidity.

Hypotheses: Enhanced cortisol generation by 11 β HSD-1 is involved in reprogramming in SGA children, causing loss of catch-up growth and metabolic morbidity. GH treatment will inhibit 11 β HSD-1 activity, improve growth and prevent metabolic changes

Patients / Methods: Twenty SGA children (birth weight < -2SD) with short stature (current height \leq -2.5 SD; 11 boys, 9 girls) age 7.1 \pm 1y (mean \pm SD), BMI 14.9 \pm 1.3 kg/m², were studied before and on GH therapy, and compared to 12 normal age-matched controls (4 boys, 8 girls), age 8.2 \pm 1.9y, BMI 16.5 \pm 2.4 kg/m². To evaluate 11 β HSD-1 activity, we developed a novel test of cortisol generation by 11 β HSD-1. After overnight suppression of endogenous cortisol by dexamethazone (Dex, 1mg/m²), children received at 8 am 25mg/m² cortisone acetate, and serum cortisol levels were measured at 0', 120', 240'. After three months of GH treatment, while IGF1 increased from 105 \pm 71 to 150 \pm 82 ng/mL (p < 0.02), the test was repeated. Gas Chromatography/ Mass Spectrometry (GCMS) evaluated urinary activity of 11 β HSD-1 as the ratio of (THF+ allo THF) / THE.

Results: Dex suppressed serum cortisol to 27 and 13 nmol/L in SGA and controls, respectively. SGA children had max. cortisol generation of 911 \pm 371 vs. 704 \pm 218 nmol/L in controls (p < 0.03). GH therapy for 3 months reduced and normalized cortisol generation to 747 \pm 251 nmol/L (p < 0.02). In SGA children urinary (THF+allo THF) / THE decreased on GH treatment from 0.70 \pm 0.24 to 0.56 \pm 0.16 (p < 0.012). In SGA, but not in control children, cortisol generation correlated negatively with birth weight (r = -0.55) and gestational age (r = -0.53) but not with sex, height, BMI, age, or metabolic markers.

Conclusions: SGA is associated with enhanced 11 β HSD-1 activity, providing excess cortisol in correlation with short gestation, low birth weight and underweight. This may be implicated in growth retardation and eventually also future metabolic morbidity. GH therapy restores normal 11 β HSD-1 activity.

Neuroendocrine Phenotype Analysis in Five Patients with Isolated Hypogonadotropic Hypogonadism Due to a L102P Inactivating Mutation of GPR54

Dr. Yarden Tenenbaum-Rakover^{1,2} Dr. Monique, Monique Commenges-Ducos³ Dr. André Iovane⁴ Dr. Chantal Aumas⁴ Dr. Osnat Admoni¹ Prof. Nicolas, Nicolas de Roux^{4,5}

¹*Pediatric Endocrine Unit, Ha'Emek Medical Center, Afula, Israel*

²*Technion Faculty of Medicine, Haifa, Israel*

³*Maternité Pellegrin, CHU Bordeaux, 33076 Bordeaux, France*

⁴*INSERM U690. Hôpital Robert Debré. 75019 Paris, France*

⁵*Faculté de Médecine Paris Sud, 94275 Le Kremlin Bicêtre, France*

Introduction: Loss of function of the G-protein-coupled receptor of kisspeptins (GPR54) was recently described as a new cause of isolated hypogonadotropic hypogonadism (IHH). In-vivo studies performed in several species have confirmed the major role of kisspeptins in neuroendocrine regulation of the gonadotropic axis and therefore sexual maturation. The aim of the present study was to specify the exact contribution of kisspeptins and GPR54 to the initiation of puberty in humans.

Patients / Methods: Detailed neuroendocrine descriptions were performed in five IHH patients bearing a new GPR54-inactivating mutation

Results: A homozygous mutation (T305C) leading to a leucine substitution with proline (L102P) was found in the five affected patients. The absence of IP accumulation under Kp10 challenge in cells expressing the L102P-mutated receptor shows that the L102P substitution completely blocks the capacity of GPR54 to activate the PLC pathway. Cell-surface binding analysis revealed normal affinity of the L102P receptor for Kp10 and a small decrease in cell-surface expression. These results indicate that an amino-acid substitution within the first extracellular loop blocks the normal conformational change of the receptor during activation as proposed for other G-protein protein-coupled receptors. Phenotypic analysis revealed variable expressivity in the same family, either partial or complete gonadotropic deficiency. LH pulsatility analysis showed peaks with normal frequency but low amplitude. Repeated GnRH tests performed between 12 and 21 y of age in one affected male revealed progressive changes in pituitary response from an early pubertal to an almost full pubertal pattern. Double GnRH test stimulations performed at a 120-min interval showed reduce dynamic pituitary response in GPR54-mutated patients.

Conclusions: GPR54 inactivation does not impede neuroendocrine onset of puberty; rather, it delays and slows down pubertal maturation of the gonadotropic axis. The L102P loss of function mutation in GPR54 results in a more quantitative than qualitative defect of gonadotropic axis activation.

Endocrine care of transsexual men in Israel: a longitudinal study

Dr. Yona Greenman¹ Prof. Naftali Stern¹

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

Introduction: In this study we describe demographic, anthropometric, metabolic and social characteristics of female to male transsexuals at baseline and during cross sex hormonal therapy.

Patients / Methods: We followed 20 transsexual subjects (mean age 26.7 ± 1.3 years; range 19-39 years) for 16.3 ± 2.6 months (range 1- 45 months).

Results: On physical examination 5 patients had mild to moderate hirsutism, without other signs of hyperandrogenism. All but one subject reported regular menses and had normal pelvic ultrasound before treatment initiation. One patient with sonographic features of PCO did not have clinical or laboratory evidence for androgen excess. Treatment consisted of IM testosterone enanthate injections once every 2 or 3 weeks in 19 patients and transdermal testosterone in one subject. Four subjects received thyroxine replacement for primary hypothyroidism, and two patients were on SSRIs for depression.

Mean weight of 68.5 ± 5.4 kg at baseline increased to 72 ± 5.9 after one year of testosterone therapy ($p = 0.0032$). All patients were normotensive and there were no changes in systolic or diastolic blood pressure during treatment. Mean total cholesterol, LDL cholesterol and triglycerides were 173 ± 8.6 mg/dl, 103 ± 8.9 mg/dl and 81 ± 9 mg/dl respectively at baseline and did not change significantly during treatment. In contrast, there was a significant decrease in HDL cholesterol levels from 53 ± 2.9 mg/dl at baseline to 44.5 ± 3.1 mg/dl after one year of hormonal treatment ($p < 0.0001$). Another prominent result of testosterone therapy was an increase in hematocrit from 38.3 ± 0.7 to 44.1 ± 1.1 after one year ($p < 0.0001$).

All subjects were single, although three were engaged in stable conjugal relationships that involved care of partner's children. Eleven subjects had completed 10-12 years of school. The remaining 9 were attending or had completed college education. Eight subjects had served in the Israeli Defense Forces as female soldiers. All patients were employed in temporary or stable work places and none lived on social security. Seven subjects were current and five past smokers, none were using illicit drugs and none had a past or present history of prostitution. There were no serious adverse events during therapy. Minor complications included acne in three patients and pain in the site of injection in one subject.

Conclusions: In conclusion, transsexual men in this cohort had normative behavior in the society and received cross sex hormone therapy without significant complications.

Molecular Mechanisms For Activation Of The Antioxidant Response Element Transcription System By Dietary Compounds

Mrs. Hagar Salman¹ Mrs. Yaara Amosi¹ Prof. Michael Danilenko¹ Prof. Joseph Levy¹ Prof. Yoav Sharoni¹

¹*Faculty of Health Sciences, Ben-Gurion University of the Negev and Soroka Medical Center of Kupat Holim, Beer-Sheva, Israel*

Introduction: Activation of the transcription factor Nrf2 and the antioxidant response element (ARE) transcription system regulates expression of phase II enzymes. These enzymes protect against damage of electrophiles and reactive oxygen intermediates. Induction of phase II enzymes is a major cellular strategy for reducing the risk of cancer, inflammation and chronic degenerative diseases. Under un-stimulated conditions, Nrf2 is bound to its cysteine-rich partner Keap1 which represses Nrf2 activity. Inducers of Nrf2 are diversified in their chemical structure, however, they are all chemically reactive electrophiles which can interact with SH groups in proteins such as Keap1 thus interfering with the inhibitory activity on Nrf2. Hydrophobic carotenoids such as lycopene, which lack any electrophilic group, were recently found by us to be potent activators of the ARE transcription system. We also showed that carotenoid oxidation products, which do contain electrophilic groups, mediate the effect of carotenoids in this activation. The aim of this study is to determine if activation of the Nrf2/ARE transcription system by carotenoid derivatives is mediated by formation of high molecular weight Keap1 complexes and to compare it to activation by other dietary compounds.

Patients / Methods: We used Western blot analysis under reducing conditions to demonstrate that in response to electrophiles, Keap1 forms complexes with a molecular weight of 150-220 kDa in T47D breast cancer cells and HEK293 cells overexpressing Keap1. In order to test whether these complexes are related to Keap1 degradation we pretreated the cells with the proteasome inhibitor (MG-132) or the lysosome inhibitor (bafilomycin).

Results: Carotenoid derivatives that activate ARE caused formation of Keap1 complexes which do not involve S-S bonds. Derivatives that had no effect on ARE did not form these complexes. Keap1 quantity and formation of complexes did not change in response to treatment with proteasome or lysosome inhibitors.

Other dietary compounds, such as the polyphenol curcumin, activated ARE and caused the formation of Keap1 complexes, whereas others, such as the polyphenol carnosic acid and the isothiocyanate sulforaphane, activated ARE but had no effect on Keap1 molecular weight under reducing conditions.

Conclusions: Activation of Nrf2/ARE transcription system by carotenoids does not involve Keap1 degradation, but probably a conformational change which causes the release of Nrf2 and allows it to enter the nucleus and transactivate ARE. It appears that different dietary compounds activate this transcription system by different mechanisms, some of which involve the formation of Keap1 complexes.

Interactions between androgen receptor and BRCA1 in regulation of IGF-I receptor gene expression in prostate cancer cells

Hagit Schayek¹ Kathy Haugk² Dr. Lawrence True³ Prof. Stephen R. Plymate²
Prof. Haim Werner¹

¹*Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv*

²*Departments of Pathology and Medicine, University of Washington, Seattle*

³*Veterans Administration Puget Sound Health Care System-Geriatric Research Education and Clinical Center, Seattle*

Introduction: The progression of prostate cancer from an organ-confined, androgen-sensitive disease to a metastatic one is associated with dysregulation of androgen receptor (AR)-regulated target genes and with a decrease in insulin-like growth factor-I receptor (IGF-IR) expression. The molecular mechanisms that are responsible for regulation of the IGF-IR gene in prostate cancer, however, remain largely unidentified. The BRCA1 gene encodes a phosphorylated transcription factor whose mutation was correlated with the appearance of breast and ovarian cancer at very young ages. Previous studies have suggested that BRCA1 functions as an AR coregulator and plays a positive role in androgen-induced cell death. In addition, linkage studies have correlated BRCA1 with a familial history of prostate cancer. The aim of our research was to assess the potential functional interactions between AR and BRCA1 in transcriptional control of the IGF-IR gene.

Patients / Methods: To this end we employed the P69 cell line, a benign primary prostatic cell line with high levels of IGF-IR, and its metastatic derivative, M12, with low IGF-IR levels. P69 and M12 cells were stably transfected with a wild type BRCA1 expression vector (or empty pcDNA3) and BRCA1 mRNA expression was assessed by qRT-PCR.

Results: Results of Western immunoblots showed that IGF-IR levels were significantly reduced in BRCA1-expressing P69 cells whereas no changes were seen in M12 cells. In addition, proliferation assays showed that BRCA1-overexpressing P69 and M12 cells display a significantly enhanced proliferation rate in comparison to empty vector-transfected cells. Consistent with these results, we have shown in two tissue microarrays including 400 individual prostate cancer patients elevated immunoreactive BRCA1 levels ($p < 0.001$). Next, BRCA1-overexpressing (or pcDNA-transfected) P69 and M12 cells were transiently transfected with an IGF-IR promoter-luciferase reporter construct, along with an AR expression vector (or empty vector) followed by dihydrotestosterone (DHT) treatment for 24 h. Results of coexpression experiments showed that the stimulatory effect of DHT on IGF-IR promoter activity was almost two-fold enhanced in BRCA1-expressing M12 cells compared to BRCA1-expressing P69 cells, suggesting that the transformation stage of prostate cancer cells has a significant impact on the interplay between AR and BRCA1 in regulation of the IGF-IR gene.

Conclusions: In summary, our results suggest that BRCA1 has a paradoxical stimulatory effect on the proliferation rate of prostate cancer cells, associated with reduction in the basal IGF-IR promoter activity and endogenous IGF-IR protein levels. Current studies are addressing the physical nature of these complex interactions.

Impaired Insulin Signaling is Associated with Decreased Incidence of Experimental Skin Cancer

Mrs. Jenny Russ¹ Dr. Weingarten Galina¹ Junji Takeda² C. Ronald Kahn³
Efrat Wertheimer¹

¹*Department of Pathology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.*

²*Dermatology, Osaka University Medical School, Osaka, JAPAN.*

³*Joslin Diabetes Center Harvard Medical School, Boston, MA.*

Introduction: It has been shown that diabetes mellitus is associated with an elevated cancer risk. This increased risk is attributed to the hyperinsulinemia associated with the pre-diabetic- and early diabetes stages. This suggests that over-activation of insulin signaling might lead to cellular transformation, or, respectively, disruption of insulin signaling might result in reduced carcinogenic potential.

Moreover, we have previously demonstrated that insulin plays a direct role in normal skin turnover, namely the balance between proliferation, differentiation and cell death. Thus, disruption of insulin signaling might lead to imbalance between these processes and contribute directly to skin carcinogenesis.

Patients / Methods: Therefore, we hypothesized that insulin signaling via Insulin Receptor (IR) is directly involved in skin carcinogenesis. To test this hypothesis, skin-specific IR knockout mice (SIRKO) were generated. In this model, disruption of IR expression is restricted to the epidermal layer of the skin. Skin tumors were induced following the standard chemical carcinogenesis protocol according to the initiation-promotion carcinogenesis model.

Results: We examined the kinetics of tumor formation (tumor multiplicity and incidence), histopathology and expression of specific epidermal markers, and found that lack of IR expression in skin resulted in a marked decrease in tumor induction efficiency and tumor proliferation capacity. Next we studied which carcinogenesis stage was disrupted by the ablation of the IR. While there was no difference in the initiation stage, the promotion stage of the carcinogenesis was inefficient in SIRKO mice compared to the control group.

In order to reveal the mechanism underlying the decreased carcinogenic potential of the skin associated with the lack of IR, we followed the turnover rate of the epidermis, and found that the exit of keratinocytes from the basal layer was accelerated in SIRKO epidermis.

Conclusions: This suggests that in the SIRKO epidermis, the initiated cells do not remain in the tissue for the sufficient time period in order to undergo the molecular events necessary for the promotion stage of skin carcinogenesis. Our data provide evidence that complete ablation of the IR in epidermis reduces tumor potential of the skin.

The Role of NDRG1 in Human Prostate Cancer

Tina Napso ¹ **Dr. Fuad Fares** ¹

¹*Department of molecular Genetics, Carmel Medical Center and the Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 34362, Israel*

Introduction: NDRG1 is a member of the N-myc down-regulated gene family. This gene was previously identified as an up-regulator of cellular differentiation, and was found to be down regulated during colon, breast and prostate tumor progression. NDRG1 is regulated by several factors including androgen, p53 and N-myc, but the biological function of NDRG1 and the physiological relevance of its role in the cellular context remain elusive. To clarify the functional role of NDRG1 in prostate cancer cells, we over-expressed the NDRG1 in three prostate cancer cell lines with different differentiation levels. Our hypothesis was that the over-expression of NDRG1 in the cells will produce differentiation in the cancer cells or may even induce apoptosis.

Patients / Methods: Normal trophoblast cells were used in order to clone the NDRG1 cDNA. The coding sequence of NDRG1 was amplified by PCR and cloned into the eukaryotic expression vector, PCDNA 3.1. In the present study human prostate cancer cell lines were used; LNCaP (well differentiated), DU145 (moderately differentiated) and PC3 (poorly differentiated).

Expression of NDRG1 was detected in the transcription and translation levels. Total mRNA was isolated from the cells and reverse transcribed for cDNA. NDRG1 was amplified by PCR reaction and then fully sequenced. In the translation level, NDRG1 protein was tested by western blotting using specific polyclonal Ab against human NDRG1. The cDNA of NDRG1 was cloned into the eukaryotic expression vector PCDNA 3.1 and transfected into the cell human prostate cancer. Differentiation was tested by detecting the differentiation factors p21 and cytokeratin 8/18 by western blotting.

Results: All the three cell lines expressed NDRG1. High levels were detected in poorly and moderately differentiated whereas low levels were found in the well differentiated cell line. The cDNAs from all cell lines were used as templates for amplification of the NDRG1 by PCR. Sequencing results of NDRG1 showed that there are no mutations in the coding sequence of the gene.

To investigate the role of NDRG1 in the prostate cancer cell lines, the cells were transfected with mock vector and with NDRG1 expression plasmid. The expression of NDRG1 in the cloned cells was tested by RT-PCR and further confirmed by Western blot.

The NDRG1 transfected cells were examined for the rate of proliferation in comparison to mock transfections. The results revealed that there were no significant differences from the control cells. However, it was found that over-expression of NDRG1 up-regulated p21 and c8/18, therefore causing or progressing differentiation in cancer cell lines.

Conclusions: Experiments conducted in this study provide evidence that overexpression of NDRG1 up regulated the differentiation markers, p21 and C8/18 of human prostate cancer cells. However, NDRG1 failed to induce cell cycle arrest and has no effect on cell proliferation rate and DNA synthesis of human prostate cancer cell lines; PC3, DU145 and LNCaP.

Furthermore, we show that NDRG1 induced p21 and C8/18 expression in a p53-independent pathway, without affecting cell cycle progression, proliferation rate and DNA synthesis.

The Role of the Antioxidant Response Element Transcription System in the Enhancement of Vitamin D-Induced Differentiation of Leukemic Cells

Mrs. Irene Bobilev¹ Ms. Victoria Rossova¹ Prof. Joseph Levy¹ Prof. Yoav Sharoni¹ Prof. Michael Danilenko¹

¹*Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University and Soroka Medical Center, Beer-Sheva*

Introduction: 1,25-dihydroxyvitamin D(3) (1,25D3) is a powerful differentiation agent, which has potential for treatment of myeloid leukemias and other types of cancer, but it induces hypercalcemia at pharmacologically active doses. We have shown that plant-derived polyphenols, such as carnosic acid (CA), markedly potentiate the differentiating action of 1,25D3 in HL60 human myeloid leukemia cells. Since polyphenols are known to be antioxidants, we hypothesize that CA can modulate certain redox-sensitive transcription systems (e.g. antioxidant response element (ARE) or activating protein-1 (AP-1) systems). This may result in upregulation of vitamin D receptor (VDR), leading to its enhanced differentiation activity. The goal of this study was to investigate the effects of CA and 1,25D3 on the ARE transcription system and to determine its involvement in differentiation of myeloid leukemia cells.

Patients / Methods: To assess ARE transactivation, U937 cells were transiently transfected with luciferase reporter constructs containing ARE sequences from NAD(P)H-quinone oxidoreductase (NQO1) or gamma-glutamylcysteine synthetase (GCS) genes. Induction of proteins was determined by Western blotting. To estimate the role of the ARE system in CA/1,25D3-induced differentiation, U937 and HL60 cells were stably transfected with vectors expressing the major ARE activating transcription factor, Nrf2, or its dominant-negative mutant (dnNrf2).

Results: We found that CA alone induced strong concentration-dependent ARE transactivation in transiently transfected U937 cells. CA treatment increased the protein level of GCS and glutathione synthesis. The latter effect was enhanced by 1,25D3. Overexpression of the wild type Nrf2 resulted in a significant increase in the differentiation-enhancing effect of CA, depending on the cell line tested. On the other hand, the expression of dnNrf2 resulted in a dramatic reduction in the expression of ARE-dependent gene products and in a significant inhibition of the differentiation induced by the CA/1,25D3 combination. This may be due to the observed significant reduction in VDR protein levels. Interestingly, the dnNrf2 transfectants also demonstrated reduced levels of proteins of the AP-1 complex, such as c-Jun and ATF2. These data suggest: 1) that the Nrf2/ARE system is one of the upstream regulators of AP-1; and 2) that AP-1 along with the Nrf2/ARE system is involved in the differentiation enhancing effects of polyphenols.

Conclusions: Our findings indicate that plant polyphenols, particularly CA, may potentiate leukemia cell differentiation induced by low concentrations of 1,25D3 via activation of the Nrf2/ARE and probably other redox-sensitive transcription systems.

Incidences and age at disease onset of Type 1 Diabetes Mellitus among Israeli Ethiopians are correlated with the duration of exposure to a new environment

Dr. Rafit Drori¹ Dr. Anat Jaffe¹

¹*Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera*

²*On behalf of the Israel IDDM Registry Study Group*

Introduction: The Israeli Ethiopian community has a high prevalence (25.2%) of the allele DRB1*0301 which is associated with increased susceptibility for Type 1 Diabetes Mellitus (T1DM). The same allele is equally prevalent in the Israeli Yemenite community (23.7%), known to have the highest prevalence of T1DM in Israel. We anticipated that the annual incidence rate of T1DM in Israeli Ethiopians would increase within few years of exposure to the new environmental conditions following the model of the Yemenite immigration. The objectives of this study were to determine trends in the incidence of T1DM among the Ethiopian population in relation to age of onset and years of environmental exposure.

Patients / Methods: Data of T1DM incidence in patients 0-17 years old of Ethiopian origin (immigrants or Ethiopian-born father) and of non-Ethiopian Israelis (NEI), between the years 1997-2005, were retrieved from the Israel IDDM Registry Study Group at the Israel Center for Disease Control (ICDC). The ICDC is a population based anonymous registry. New cases are reported by all the centers for T1DM in Israel. Collected demographic data included place of birth, year of immigration, and age at disease onset.

Results: The incidence rates of T1DM among children of Ethiopian origin and of NEI during the study period increased from 9.5/100,000 and 7.4/100,000 in 1997, respectively, to 21.7/100,000 and 11.4/100,000 in 2005.

Of 49 T1DM Ethiopians with newly diagnosed T1DM, 39 (81%) were born in Israel and 9 (19%) emigrated from Ethiopia. 1-missing information. The average age of disease onset in the two groups was 8.0 ±3.9, and 10.7 ±3.4 years, respectively. Year of immigration was documented in 6 patients of the immigrating group. In that group, the average time interval from the immigration date to disease onset was 7.5 ±3 years.

Conclusions: There is a trend for increase in T1DM incidence in Israeli children. The incidence of T1DM among children of Ethiopian origin is higher than the incidence in the NEI population. The equivalence of the time interval between immigration date and the onset of disease, and the average age at onset among the Israeli born Ethiopians, may reflect an accumulating environmental exposure.

Novel de novo heterozygous mutation in SUR1 presenting as hyperinsulinism (HI) in infancy followed by overt diabetes in adolescence – clinical and molecular studies

Dr. Maha Atwan¹ Mr. Jeremy Bushman² Dr. Show-Ling Shyng² Mrs. Avital Perry¹ Mrs. Sharona Tornovsky babaey¹ Prof. Benjamin Glaser¹ Dr. David Zangen¹

¹*Hadassah Hebrew University Medical Center Jerusalem*

²*Oregon Health and Science University, Portland, USA*

Introduction: Neonatal HI causes hypoglycemic episodes, often resulting in neurological sequelae and impaired glucose-stimulated insulin secretion (GSIS) later in life. Dominant and recessive inheritance due to mutations in several β cell genes (e.g. SUR1, Kir6.2) can cause HI as well as impaired insulin secretion.

Patients / Methods: We describe a 10.5 year old female who presented with new onset diabetes mellitus, fasting blood glucose of 220 mg/dl and HbA1C of 10.6%. She was born LGA (5 Kg) to a non-diabetic mother. Until the age of 3y she had frequent episodes of hypoglycemia treated with intravenous glucose and oral sweets, resulting in obesity since infancy. At 1y of age she developed convulsive disorder that is partially controlled medically. Currently, she is fully pubertal, obese (BMI 30.2 kg/m²), has aggressive behavior with poor school performance. Glucose levels were >200mg% throughout 72 hours of continuous subcutaneous glucose monitoring, with a low insulin secretion during IVGTT. Anti-insulin and anti-GAD antibodies were negative and fatty acid metabolites and ammonia were normal. Brain CT was normal. Insulin dose requirements and glucose levels were typical of type 2 diabetes.

Results: A novel de novo heterozygous missense SUR1 mutation (R370S) was identified in the patient's DNA but not in that of either parent (paternity/maternity confirmed genetically). Co-transfection of Kir6.2 and mutant SUR1 in COSm6 cells demonstrated channel expression on the cell membrane but only minimal current when exposed to MgADP (<10% of WT). The mutant channel was unresponsive to diazoxide but surprisingly did respond to glibenclimide. When cells were made ("heterozygous") by expressing both WT and mutated protein to imitate our clinical setting, the MgADP-induced current was reduced by 50% of WT. The "heterozygous" cells responded partially to diazoxide and again were responsive to glibenclimide. Based on the glibenclimide in-vitro response the patient has been treated with repaglinide and glibenclimide and preliminary data show a good clinical response.

Conclusions: We describe a patient with HI due to a novel de-novo mutation. The manifestation of overt diabetes during adolescence and not only mildly impaired insulin secretion during remission from HI is unique. The expression studies of the mutated SUR1 in both the homozygote and heterozygote states confirm channel dysfunction and indicate a possible mechanism for HI in this and other heterozygote cases. Furthermore, the in-vitro channel responsiveness to glibenclimide suggests that this drug may be therapeutically useful in this patient.

PKC epsilon is involved in the control of insulin biosynthesis and secretion in pancreatic beta-cells

Mr. Nasim Warwar¹ Dr. Esther Haber¹ Ms. Avital Dov¹ Mrs. Eva Abramovitch¹
¹ Prof. Erol Cerasi¹ Dr. Rafael Neshet¹

¹*Endocrinology and Metabolism Service, Department of Medicine, Hadassah - the Hebrew University Medical Center, Jerusalem*

Introduction: Glucose metabolism affects most major signal pathways in pancreatic beta-cells including insulin production, storage and exocytosis. Multiple protein kinases mediate these effects; however, the role of most is poorly defined. Here we examined the dynamics of glucose dependent activation and translocation of PKC isoenzymes in beta-cells of normal and diabetic rats. Of the six isoforms followed, the role of PKCepsilon in the control of insulin secretion and biosynthesis was further explored.

Patients / Methods: (A) Isolated pancreata from Wistar and GK rats were stimulated with glucose (16.7 mmol/l) and fixed at time points selected to reflect the biphasic dynamics of insulin release. Sections were immunostained with anti-PKC isoenzymes antibody, counterstained with anti-insulin antibody and imaged using a three-gun laser-scanning confocal microscope.

(B) Adenoviral vectors expressing a translocation inhibiting peptide or kinase dead enzyme, were used to inhibit PKCepsilon's translocation or to inhibit the enzyme's activity, respectively. siRNA constructs were used to diminish PKCepsilon levels. Isolated islets were infected or transfected and used in perfusion studies or in batch studies to follow total protein and insulin biosynthesis.

Results: Glucose stimulus resulted in the concentration of PKCepsilon near the nucleus, strongly associated with insulin staining and with a dynamics resembling that of biphasic insulin response. However the signal persisted for 15 min after cessation of stimulation. Adenovirus- or siRNA-mediated depletion of PKCepsilon function led to partial inhibition of insulin secretion in isolated islets. No glucose-induced translocation of PKCepsilon was observed in GK rats pancreas, suggesting a role in the defective insulin response in this model for T2DM. Beta-COP-1 was identified as the anchoring protein for PKCepsilon. The fact that beta-COP-1, is a member of the COP complex that controls Golgi protein transport, led us to examine the role of PKCepsilon in production of beta-cell proteins in general and that of insulin specifically. In isolated islets, diminished PKCepsilon activity by adenovirus or by siRNA, lead to specific inhibition of proinsulin biosynthesis and its conversion to insulin.

Conclusions: Our data suggest a role for PKCepsilon in insulin biosynthesis and in granules exocytosis in pancreatic beta-cells. Its diminished activity might be one of the defects in stimulus-secretion coupling in the diabetic islet.

Rapamycin Treatment Exacerbates Nutrition-Induced Diabetes in *Psammomys obesus*

Dr. Merav Fraenkel¹ Dr. Mali Ketzinel Gilad¹ Mrs. Yafa Ariav¹ Ms. Melis Karaca² Mr. Julien Castel² Dr. Christophe Magnan² Prof. Erol Cerasi¹ Prof. Nurit Kaiser¹ Dr. Gil Leibowitz¹

¹*Hadassah medical center, Hebrew university, Jerusalem*

²*Universite Paris, Paris, France*

Introduction: mTOR is a serine threonine kinase regulated by nutrients and growth factors. Activation of mTOR/S6K1 results in downregulation of IRS1 and IRS2 with subsequent reduced Akt phosphorylation, hence insulin resistance. It was suggested that inhibition of mTOR/S6K1 could become a therapeutic target in insulin resistant states, such as obesity and Type 2 diabetes. We tested this hypothesis in the *Psammomys obesus* (*P. obesus*) model of diet-induced Type 2 diabetes using the mTOR/S6K1 inhibitor rapamycin.

Patients / Methods: Diabetic *P. obesus* fed the high-energy (HE) diet were treated with 0.2 mg/kg/day (IP) rapamycin or vehicle for 14 days. The effects of rapamycin on the metabolic state of the animals were assessed by measuring body weight, blood glucose, serum lipids and by IPGTT. Insulin sensitivity was determined by the intraperitoneal insulin tolerance test. Serum samples were analyzed for the level of insulin, triglycerides, FFA and ketone bodies. Insulin signaling was assessed in insulin target organs and in isolated islets.

Results: Rapamycin therapy accelerated diabetes in *P. obesus*. At 2 weeks, blood glucose of the rapamycin treated animals was 436±26 mg/dl vs 303.3±55.6 mg/dl in the control group ($p<0.001$). This was accompanied by weight loss. Body weight of the rapamycin-treated animals was 174.4±4.7 gr vs 200.3±4.4 gr that of control animals ($p<0.001$). Hyperglycemia in diabetic *P. obesus* was accompanied by a 10-fold increase of serum insulin compared to normoglycemic controls, while rapamycin therapy completely abolished this increase. The hypoinsulinemia in the rapamycin treated *P. obesus* was accompanied by a 55-, 12- and 23-fold increase in serum triglycerides, FFAs and ketone bodies, respectively ($p<0.001$). Rapamycin completely abolished S6 kinase and reduced IRS1 serine 636/639 phosphorylation. However, this was associated with reduced total IRS1 levels and Akt phosphorylation at serine 473 in muscle, fat and liver. In line with this observation, intraperitoneal insulin tolerance test revealed a lower insulin sensitivity of the rapamycin-treated *P. obesus* compared to controls. In the islets, rapamycin therapy increased IRS2 expression *in vivo* and *in vitro*. However, this was not associated with increased Akt phosphorylation. Moreover, rapamycin therapy resulted in a 3-fold decrease in β -cell mass and impaired glucose-stimulated insulin secretion and proinsulin biosynthesis.

Conclusions: We conclude that treatment of diabetic *P. obesus* with rapamycin generates a fulminant form of diabetes characterized by excessive hyperglycemia, weight loss, hyperlipidemia and hypoinsulinemia. Exacerbation of diabetes results from both increased insulin resistance, reduced β -cell mass and impairment of β -cell function. These findings make it unlikely that mTOR/S6K1 inhibition is an adequate therapeutic approach to insulin-resistant diabetes.

Abstract Code: A20

The role of Thioredoxin interacting protein in β -cell dysfunction in Type 2 diabetes

Miss Maayan Shaked¹ Miss Ety Bachar¹ Prof. Erol Cerasi¹ Prof. Nurit Kaiser¹
Dr. Gil Leibowitz¹

¹*Endocrinology and Metabolism Service, Department of Internal Medicine, Hadassah - Hebrew University Medical Center, Jerusalem*

Introduction: Thioredoxin (TRX) is an important antioxidant that is highly expressed in pancreatic β -cells. The expression of thioredoxin-interacting protein (Txnip), the endogenous inhibitor of TRX is induced by glucose. We hypothesize that Txnip is an important mediator of glucose deleterious effects on β -cell function in Type 2 diabetes. Moreover, it was recently shown that Txnip is regulated by NO, which can potentially exert oxidative stress on the pancreatic β -cells. We, therefore, studied the effect of nNOS inhibition on Txnip expression and β -cell function.

Patients / Methods: Txnip expression was studied by qPCR and Western blot in islets of diabetic *Psammomys obesus* (P. obesus) and in vitro following chronic exposure of P. obesus islets and INS1e β -cells to high glucose. To further study the role of Txnip in β -cell dysfunction in response to hyperglycemia, we performed siRNA knockdown of Txnip and analyzed its effects on insulin secretion. TRX activity was measured by the insulin disulfide reduction assay. Glucose-stimulated insulin secretion was studied by static incubations followed by RIA.

Results: Txnip expression was increased in islets derived from diabetic P. obesus and in response to high glucose in vitro. This was accompanied by decreased thioredoxin activity and glucose-stimulated insulin secretion. Preliminary studies showed that siRNA knockdown of Txnip in INS-1e cells improved insulin secretion after chronic exposure to high glucose. Moreover, inhibition of nNOS by L-NAME augmented the induction of Txnip by glucose, suggesting that nNOS is a negative regulator of Txnip in the pancreatic β -cells. Chronic treatment of INS-1e-cells exposed to chronic hyperglycemia with L-NAME failed to improve insulin secretion, probably due to increased Txnip expression.

Conclusions: Txnip expression is increase by high glucose and nNOS inhibition resulting in reduced TRX activity. Inhibition of Txnip activity might improve glucose-stimulated insulin secretion in β -cells exposed to high glucose. Therefore, inhibition of Txnip may be a therapeutic target for amplifying insulin secretion and for protecting pancreatic islets from the deleterious effects of chronic hyperglycemia in Type 2 diabetes.

Dual Treatment Of The Prostate Cancer Cell Line DU145 With The Anti-Egfr Antibody And 1,25-Dihydroxyvitamin D3 Efficiently Suppresses Cancer Cell Growth

Dr. Vladimir Gavrilov¹ Dr. Olga Belochitski² Prof. Samuel Ariad² Mr. Vladimir Fridman¹ Prof. Shraga Shany¹

¹*Department of Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University of the Negev*

²*Department of Oncology, Soroka Medical Center and Faculty of Health Sciences, Ben-Gurion University of the Negev*

Introduction: The development of androgen resistance is a common outcome in patients with advanced prostate cancer (PCa), and results in fewer therapeutic options and limited survival for patients. The progression from hormone-dependent to hormone-independent prostate cancer disease is accompanied by activation of the epidermal growth factor receptor (EGFR) family and its crosstalk with androgen receptor pathways. Therefore, targeting the EGFR pathway is a promising treatment strategy for aggressive androgen-refractory PCa. Since the active metabolite of vitamin D also affects the EGFR-pathway by decreasing EGFR mRNA expression which is followed by reduced synthesis of the EGFR, the goal of the present study was to enhance the effect of the anti-EGFR antibody (cetuximab) by combining it with 1,25(OH)₂D₃.

Patients / Methods: Androgen-refractory PCa cell line DU145 was grown in RPMI-1640 medium containing 10% FCS. Cancer cells were treated during 4 days with 5 nM cetuximab or 100 nM 1,25(OH)₂D₃, or with their combination. The effect of the suggested treatment on DU145 cells proliferation, cell cycle and apoptosis was evaluated by crystal violet test, propidium iodide FACS procedure, and APOPercentage kit of Biocolor Assay firm, respectively.

Results: Single-drug treatment with cetuximab or 1,25(OH)₂D₃ decreased DU145 cell growth by approximately 25% and caused a 1.5-1.7-fold increase of apoptosis but did not affect cell-cycle distribution. However, combined treatment inhibited DU145 cell proliferation by 40%, caused a considerable cell-cycle arrest in G₀-G₁ phase and enhanced apoptosis by 2.5-fold (compared to the control, $p < 0.0001$, $p < 0.006$ and $p < 0.0001$, respectively).

Conclusions: Presented results support our hypothesis that targeting the EGFR-pathway with a combination of the anti-EGFR antibody and the active metabolite of vitamin D might be effective in combating PCa and could provide the basis for clinical application in the treatment of hormone-refractory PCa. Furthermore, this combination of drugs could be used as a neoadjuvant therapy for DNA-damaging drugs since inhibition of the EGFR pathway results in decreased DNA-dependent protein kinase activity and consequently reduced DNA repair.

Mechanism underlying the relationship between FSH, inhibin B (INB), anti-Mullerian hormone (AMH), and testosterone (T) secretion during long term treatment with Gonadotropin releasing-hormone agonist (GnRHa) in prostate cancer

Dr. Talia Eldar-Geva¹ Dr. Gad, Gad Liberti¹ Dr. Boris Chertin¹ Dr. Alon Fridmans¹ Mrs. Hadassa Hartman² Prof. Amicur, Amicur Farkas¹ Prof. Irving Spitz²

¹Shaare-Zedek Medical Center, Jerusalem

²Institute of Hormone Research, Jerusalem

Introduction: The main inhibitor of FSH secretion is INB, which is secreted by Sertoli cells (SC). During long-term GnRHa administration, LH and testosterone (T) are fully suppressed whereas FSH is only suppressed transiently. The mechanism is unknown.

Patients / Methods: We examined serum INB, FSH and AMH levels in 10 patients with prostate cancer who were evaluated before, during and following insertion of a hydrogel implant releasing 60µg of the GnRHa histrelin daily. Two weeks prior to and up to 3 months following implant insertion, the patients were treated with an antiandrogen (flutamide 750mg daily). Following implant removal flutamide was re-administered. Serum LH, T and PSA responses have been published (1).

Results: The initial FSH and INB responses were characterized by a gradual increase during antiandrogen treatment (mean±SEM; FSH from 6.4±0.8 to 9.6±1.5IU/L and INB from 95±11 to 140±24pg/mL); levels fell after implant insertion but the responses diverged; FSH levels decreased until 2 weeks post insertion and then gradually increased while INB continued to decrease. AMH levels did not change during flutamide treatment, but increased 1 month post implant insertion (from 2.3±0.8 to 7.2±2.5ng/mL) and then remained elevated for the duration of implant use. Following implant removal, FSH levels increased (from 5.7±4.1 to 10.8±2.3IU/L), AMH decreased (from 8.3±2.3 to 0.6±0.1ng/mL), while INB did not change. Significant negative correlations were found between FSH and INB on the day of implant insertion ($r=-0.790$), 12 months later ($r=-0.857$) and 1-3 months following implant removal ($r=-0.819$ to -0.863).

Conclusions: The results suggest that the secondary increase in FSH following initial suppression during prolonged GnRHa treatment might be related to impaired INB secretion. Intact SC should respond to the increase in FSH by secreting INB, producing a new steady-state. The absent INB response suggests that long-term exposure of SC to GnRHa partially impairs their function, although this appears to be selective since AMH increased. T is fully suppressed 1 month after insertion and increases after implant removal (1, 2). The AMH profile observed suggests that T regulates AMH secretion.

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2. J Urol 173:784.

Registry of Adrenocortical Carcinoma in Israel - A preliminary report on 104 patients

Jaffe A¹, Barchana M^{2,4}, Tripto Shkolnik L¹, Liphshitz I², Toledano Y¹, Hopp M³, Benbassat C^{5,7}

¹Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera;

²Israel National Cancer Registry, Ministry of health, Jerusalem;

³Tel Aviv U & HOP research;

⁴School of public Health, Haifa University;

⁵Endocrine Institute, Rabin Medical Center, Petah Tikva and ⁷Sackler School of Medicine, Tel Aviv University.

Introduction: Adrenocortical Carcinoma (ACC) is a rare and aggressive disease with a prevalence of 1-1.6 cases per million. With some patients showing a better outcome than others, the appropriate treatment is still controversial. Here we report on the current information collected by the Israel National Cancer Registry (INCR) on ACC.

Patients / Methods: Since 1982 reporting of malignancies to the INCR became mandatory in Israel. Sources to the INCR include discharge hospital letters, pathology reports, oncology databases and death certificates. Data are analyzed by INCR officers and loaded to a computerized program according to the ICD-O system. The INCR file includes coding of 46 different morphologies of the adrenal glands, all of which were reviewed for this report. Records were informative for demographical data (date & place of birth, immigration, vital and marital status and residential history) and data on disease occurrence (site & morphology, place & date of diagnosis, stage & treatment, and second primaries). There is no active follow-up on treatment and endocrine status of registered patients

Results: Between 1982-2005 there were 638 patients diagnosed with adrenal pathology reported to the INCR. They were recoded as ACC in 123 cases (17%), carcinoma, adenocarcinoma or tumor malignant NOS in 105 (16%), pheochromocytoma (13.9%), neuroectodermal tumors (32.9%), solid metastasis (1.5%), hematological metastasis (3%), adenoma (5.6%), and other (9.5%). Following a review of files, that included pathology reports, 19 patients coded initially as ACC were excluded due to equivocal data. Of the 104 patients diagnosed with ACC, 41% were male and 59% female. Mean age at diagnosis was 47.7 ± 19.3 (range 0.9-83.2). There were 6 patients younger than 10 years old. Median tumor weight was 340 grams (range, 40-4191) and median size was 10.5 cm (range, 4-30). All but one tumor were larger than 4 cm, and all but 8 were larger than 6 cm. The overall median survival was 2.3 yrs (mean 4.57 ± 5.2 ; range 0-24) and was not affected by the age of the patients or their tumor size. Patients diagnosed before (n=36) and after (n=67) year 1995 showed a similar overall survival (median 3.25 vs 2.05 yrs, p=ns)

Conclusions: The paucity of information on ACC in Israel, the tumor's highly fatal outcome, and the limited data provided by the INCR, points out on the urgent need to collaborate in a multi-professional agenda and to establish a comprehensive Israeli National ACC Registry, aiming to identify etiologic and prognostic factors and to improve the medical care of these patients.

Hospital-base registry of differentiated thyroid cancer: a useful resource to improve the clinical management of thyroid cancer patients

Dr. Carlos Benbassat¹ Dr. Dania Hirsch¹ Dr. Gloria Zvetov¹ Dr. Ilana Shraga-Zlutzky¹ Dr. Ruth Weinstein¹ Dr. Monica Gaspar¹ Dr. Hanna Bernstine²
Dr. Adam Steinmetz² Dr. Ronit Kalmanovitch¹ Dr. Joelle Singer¹, Dr. Ilan Shimon¹

¹*Endocrine Institute, Beilinson Hospital*

²*Dept of Nuclear Medicine, Beilinson Hospital*

Introduction: Population-base registries are useful tools for the analysis of incidence and survival of thyroid cancer, but the individual data on clinical features and treatment modalities needed for improving disease management is usually missing. In 2005 we started a registry of all non-medullary thyroid cancer patients followed at our institute. The preliminary data is reported.

Patients / Methods: Demographics, risk factors, medical history, diagnostic workup, primary treatment, follow-up and outcome are recorded by the attending physician in a common excel spreadsheet for all thyroid cancer patients, and the data is updated at each follow-up visit.

Results: By January 2007 there were 762 patients registered (78% female, mean age 47.6±16) with a mean follow-up of 9 years (median 5 yr). Familial disease accounted for 3.9% cases. Previous radiation exposure was reported in 9.2%. All patients were surgically treated (total thyroidectomy 88%, LN dissection 21%). Initial surgery (78% at Rabin Medical Center) was performed before 1990 in 144 patients, between 1990-2000 in 245 and between 2001-2006 in 360. Histology was available for 728 patients: 625 (83%) had PTC, including 137 patients with follicular variant, 55 (7.5%) had FTC, and 22 (3%) had HCC. Other types were insular 4, sclerosing 6, tall cell 9, columnar cell 4, anaplastic 3 patients each. The disease was microscopic in 17% and multifocal in 51% of cases. Tumor size was T1 in 57%, T2 in 19%, T3 in 19% and T4 in 5%. Distant metastases developed in 80 (11.5%) patients, 30 of them at diagnosis. Distribution by site was: lungs 78 patients, bones 25, and brain in 3; with multiple sites in 17. Initial I-131 therapy was given to 87.5% patients and repeated at least once in 32% of them. External radiotherapy was given to 47 (7.1%) patients. Anti Tg antibodies were present in 39 patients, and 49 patients were Tg positive/TBS negative. At the time of this report 11 patients were death and 22 were lost to follow-up; 72% of patients were alive without disease.

Conclusions: This database provides the setup for future studies on specific non-medullary thyroid cancer subgroups, aiming to improve the clinical management of these patients.

Differentiated thyroid carcinoma in the pediatric population – comparison between prepubertal children and adolescents

Dr. Yael Lebenthal¹ Prof. Moshe Phillip² Dr. Liora Lazar

Institute for Endocrinology and Diabetes, Schneider Children's Medical Center of Israel, Petah Tikva

Introduction: Differentiated thyroid carcinoma (DTC) is uncommon in children and adolescents. The majority of affected youngsters are diagnosed during puberty; however, the disease may also occur earlier. Since the prevalence is low in prepuberty, clinical studies tend to include younger children within the larger group of adolescents rather than analyzing them separately.

Objective: To study the clinical characteristics of 26 children (9 prepubertal and 17 pubertal) diagnosed with DTC and to determine the differences in the clinical presentation, course, and outcome between these two age groups.

Patients / Methods: The records of 9 prepubertal children and 17 adolescents diagnosed and followed in our tertiary pediatric endocrine clinic from 1985 to 2006 were reviewed. Age and pubertal stage at DTC diagnosis, extension of tumor, treatment modality, course, and recurrence rate were analyzed.

Results: The age range at diagnosis of the prepubertal and the pubertal groups were 6.1-11.5 years and 11.5-17.2 years, respectively. At diagnosis only 9 patients had disease confined to the thyroid gland (1 prepubertal). Regional lymph nodes and pulmonary metastases were present in 17 (8 prepubertal) and 9 patients (5 prepubertal), respectively. Initial treatment consisted of total thyroidectomy in all patients with nodal dissection in 13 patients (5 prepubertal). Permanent post operative complications were documented in 4 patients – vocal cord paralysis in one prepubertal patient and hypoparathyroidism in 3 patients (2 prepubertal). All patients received adjuvant therapy with (131) I followed by TSH suppression. During follow-up of a median of 4.1 years (range 0.5-21 years) all 26 patients were alive. Eleven patients (4 prepubertal) were disease free for a median of 7.9 years (range 2.5-21 years). Six patients (1 prepubertal) had persistent disease for 2.7-4 years following initial (131) I treatment despite adequate TSH suppression and additional (131) I treatment. Five patients developed recurrences (1 prepubertal) at a median time of 4 years (range 3 - 12.5 years) from initial (131) I treatment. The follow-up of the remaining 4 patients (3 prepubertal) is less than 2 years and thus insufficient to determine their clinical course.

Conclusions: Carcinoma of the thyroid has a more aggressive presentation with extensive spread of disease, and more post operative complications in prepubertal children. However, appropriate initial treatment followed by TSH suppression and meticulous surveillance, results in a clinical course and outcome similar to adolescents.

The Orientation Of The Alpha And Beta Subunit Domains In Single-Chain Bovine LH Analogs Affects The Secretion And Steroidogenic Response In Vitro

Ms. Moran Grinberg¹ Ms. Sigal Nakav¹ Ms. Svetlana Pen² Ms. Ada Dantes³
Dr. Ruth Braw-Tal² Prof. Abraham Amsterdam³ Dr. David Ben-Menahem

¹*Department of Clinical Pharmacology, The Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel*

²*Institute of Animal Science, Agricultural Research Organization, The Volcani Center, Beit Dagan 50250, Israel*

³*Department of Molecular Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel*

Introduction: The gonadotropins LH, FSH and human (h)CG are non-covalent heterodimers composed of a common alpha- and the hormone-unique beta subunit. To by-pass the assembly process of the hormone and expand the range of structure-function analyses, single-chain (SC) gonadotropins were engineered. The secretion and bioactivity of SC hCG analogs in the orientation of NH₂-beta-alpha-COOH (designated BA configuration) is similar to the heterodimer. Switching of the relative position of the subunit domains (AB format) significantly changed the conformation of the hCG tethers, and modified the secretion, receptor binding and, to a lesser extent, adenylyl cyclase activation. However, less is known as to how changing the topology affects steroidogenesis.

Patients / Methods: We used here PCR methodology to construct SC bovine LH analogs in the AB configuration with and without a linker derived from the CTP domain of the hCGbeta subunit, NH₂-alpha-CTP-LHbeta-COOH (denoted as aCTPLHb) and NH₂-alpha-LHbeta-COOH (aLHb). The secretion of these variants from stably transfected CHO cells and the effect on steroidogenesis in follicular derived cells were compared to analogs in the BA design, NH₂-LHbeta-CTP-alpha-COOH (LHbCTPa) and NH₂-LHbeta-alpha-COOH (LHba).

Results: Secretion of aLHb from CHO cells was inefficient and the yield was smaller than LHba. In contrast, the release of aCTPLHb was quantitative and more efficient than LHbCTPa, as was previously shown for analogous hCG variants. While LHba induced progesterone release from immortalized rat granulosa cells expressing the LH receptor, aLHb did not stimulate it. The CTP-spaced analogs were bioactive in this bioassay, but with different EC₅₀ values. The estimated potency for this steroid of aCTPLHb (AB orientation) was smaller by 20 fold, as compared to LHbCTPa (BA). Furthermore, while the aCTPLHb analog stimulated progesterone release from primary bovine theca cells about 3 fold less than LHbCTPa, androstenedione accumulation in the media appeared to be similar. As a result, the androstenedione/progesterone ratio for the aCTPLHb analog was significantly increased relative to LHbCTPa (2-3 fold). This unequal response suggests a distinct regulation of progesterone and androstenedione biosynthesis in bovine theca cells by these variants, by an as yet unknown mechanism.

Conclusions: The results of the experiments suggest that the incorporation of the linker is crucial for fruitful interactions between the tethered subunit domains of bovine LH analogs in the AB orientation, to establish determinants for secretion and bioactivity. Our data demonstrate major differences in steroidogenesis following stimulation of the receptor with alternative SC LH analogs.

LH-induced caspase activation in rat preovulatory follicles is coupled to mitochondrial steroidogenesis

Dr. Keren Yacobi ¹ Prof. Alex Tsafri ¹ **Dr. Atan Gross** ¹

¹*Department of Biological Regulation, The Weizmann Institute of Science, Rehovot*

Introduction: Atresia and luteolysis are well-documented processes, in which most of the growing ovarian follicles and all corpora lutea, respectively, are eliminated by apoptosis. We have previously reported that LH and FSH enhance caspase-3 and -7 activity and apoptosis in the theca-interstitial cells of rat preovulatory follicles in culture.

Patients / Methods: We have used cultured follicles to examine whether LH-induced caspase activation is related to the ability of LH to stimulate steroid production. In these studies, we used three inhibitors of enzymes involved in steroid production: aminoglutethimide and ketoconazole, acting on cytochrome P450scc located at the mitochondria, and epostane, acting on 3beta-HSD located at the endoplasmic reticulum.

Results: We found that treatment with either aminoglutethimide or ketoconazole, but not with epostane, significantly reduced LH-induced caspase-3 and -7 activation and apoptosis, suggesting the mediation of LH-induced caspase activation by P450scc. Supplementing pregnenolone, the product of P450scc catalysis, to follicles treated with aminoglutethimide, did not restore LH-induced caspase activation. On the other hand, treatment with antioxidants inhibited LH-induced caspase activation. Moreover, LH treatment was associated with an increase in reactive oxygen species (ROS) which was inhibited by aminoglutethimide. Thus, P450scc catalysis results in an increase in ROS, which in turn may trigger/facilitate caspase-3 activation. Finally, we found that in rat corpora lutea in-vivo, increase in steroidogenesis was accompanied by an increase in caspase activity.

Conclusions: Thus, this study reveals a linkage between two seemingly distinct processes in which LH-induced caspase activation in cultured rat preovulatory follicles is coupled to mitochondrial steroidogenesis via P450scc.

Endometrial-trophoblast interaction: The role of Plexin-B1 and Progesterone receptors

Mrs. Haggard Harduf¹ Dr. Shlomit Goldman² Prof. Eliezer Shalev^{1,2}

¹*Rapaport faculty of Medicine, Technion, Haifa*

²*Laboratory for research in reproductive science, Department of Obstetrics and Gynecology, Ha'Emek Medical Center, Afula*

Introduction: Human implantation involves complex interactions between the embryo and the receptive endometrium. These interactions are probably mediated by endometrial epithelium receptors. However, their role in embryo-maternal interactions remains unclear.

Patients / Methods: Two endometrial cell lines were used: HEC-1A considered to be of low receptivity and RL95-2 considered to be of high receptivity. Western blot and RT-PCR analysis were utilized to study the receptor expression profile for Plexin-B1 (PLXNB1) and progesterone receptor (PR). Attachment and growth assays of spheroids from JAR cell lines on endometrial cell lines were carried-out. In order to study the role of PLXNB1 in spheroid attachment and invasion, we used neutralizing antibodies against PLXNB1, which were added to RL95-2. In order to verify the role of PLXNB1 in embryo-endometrial interactions, HEC-1A cells were transfected with expressing vector encoding for PLXNB1.

The role of PR in spheroid attachment and invasion was studied by adding progesterone (P) to HEC-1A cells and the P antagonist RU-486 to both endometrial cell lines.

Results: PLXNB1's expression level was significantly higher in RL95-2 as compared with HEC-1A cells. The addition of neutralizing antibodies against PLXNB1 to RL95-2 cells significantly decreased the spheroid attachment rates ($25\% \pm 11$ versus $63\% \pm 7.8$, respectively, $P < 0.02$). PLXNB1 transfected HEC-1A (HEC-1A-2) cells showed a significant increase in spheroid attachment versus un-transfected cells ($66.7\% \pm 3.5$ versus $48.6\% \pm 3.7$, respectively, $p < 0.01$). Addition of neutralizing antibodies against PLXNB1 to HEC-1A-2 significantly decreased the spheroid attachment rates by 1.4 fold. Changes in the distribution of the GTPases, R-Ras and Rho-A, involved in the PLXNB1 signaling, was observed during spheroid attachment to RL95-2 cells. A differential PR profile was documented with the dominance of PRB in HEC-1A cells. Addition of progesterone to HEC-1A cells showed no effect on spheroid growth and attachment rate; however, the progesterone antagonist RU-486 increased the spheroid attachment rate to HEC-1A cells ($89.5\% \pm 1.7$ versus $75.5\% \pm 2.8$, respectively, $P < 0.001$). No change in the spheroid attachment rate to RL95-2 cells was found after treatment with RU-486.

Conclusions: We hypothesize that the expression of PLXNB1 in receptive endometrium is related to cytoskeleton remodeling, which influences the ability of endometrium to allow blastocysts to adhere. A shift in the PR profile could be proposed as one of the mechanisms involved in acquiring endometrial receptivity.

The role of *Xenopus* membrane progesterone receptor beta in mediating the effect of progesterone on oocyte maturation.

Dr. Liat Josefsberg Ben-Yehoshua¹ Ms. Andrea L Lewellyn¹ Dr. Peter Thomas²
Dr. James L Maller¹

¹*Howard Hughes Medical Institute, University of Colorado Health Sciences Center, Denver*

²*Marine Sciences Institute, University of Texas, Port Aransas*

Introduction: Rapid, non-genomic membranal effects of progesterone were demonstrated in amphibian oocytes over 30 years ago. Recently, a distinct family of membrane progestin receptors (mPR) has been cloned in fish and other vertebrate species. In this study we explore the role of mPR in promoting oocyte maturation in *Xenopus laevis*.

Patients / Methods: The expression of XmPR was verified by RT-PCR analysis of stage VI *Xenopus* oocytes. The expression and localization of XmPR beta protein, was analysed on plasma membranes as well as whole cell lysates by immunoblotting with specific antibodies raised against an N-terminal peptide of XmPR beta. Loss of function studies were performed by microinjecting oocytes prior to progesterone treatment with affinity-purified antibodies raised against two different regions of the protein. The effect of over expression of XmPR beta on oocyte maturation was examined on oocytes - microinjected with mRNA encoding Myc-XmPR beta. Characterization of progesterone binding was carried out by radioligand binding assay of XmPR beta in CHO cells stably expressing the amphibian homolog.

Results: RT-PCR analysis indicates that *Xenopus* oocytes contain transcripts for the mPR beta ortholog, similar to what has been reported in zebrafish oocytes, and Western blotting shows that the protein is expressed on the oocyte plasma membrane. Microinjection of mPR-specific antibodies into oocytes resulted a dramatic inhibition of progesterone-dependent oocyte maturation, whereas microinjection of mRNA encoding Myc-XmPR beta resulted in an accelerated rate of progesterone-induced oocyte maturation, concomitant with membranal localization of the protein. Binding studies in mammalian cells expressing XmPR beta confirmed specific binding of progesterone by the expressed protein.

Conclusions: These results suggest that XmPR beta is the physiological progesterone receptor involved in initiating the resumption of meiosis during maturation of *Xenopus* oocytes.

Elevated ET-2 In The Young Bovine Corpus Luteum: Induction By LH And HYPOXIA

Dr. Eyal Klipper¹ Mrs. Yonit Mastich¹ Prof. Dieter Schams² Prof. Rina Meidan¹

¹*Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

²*Institute of Physiology Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany*

Introduction: Endothelins (ETs; ET-1, ET-2, and ET-3) comprise a family of small peptides with diverse paracrine/autocrine actions. ET-1 and ET-2 have similar structures, differing by only two amino acids. ETs bind two receptor subtypes, ETA and ETB, which are G protein-coupled receptors. The active ETs are generated by hydrolysis of big ETs, catalyzed by ET-converting enzyme-1 (ECE-1). ET-1, the main ET produced in endothelial cells (ECs), was extensively studied and was shown to act as a mediator of luteolysis; however, little is known about ovarian ET-2. Recently ET-2 was proposed to promote ovulation in rodents by inducing follicular rupture via a progesterone-dependent mechanism.

Patients / Methods: To examine the possible role of ET-2 in a monovulatory species, we studied its pattern of expression in the bovine ovary. Initially, ET-2 mRNA was examined during folliculo-luteal transition. GnRH was administered to heifers to induce LH surge and ovulation (which takes place app. 30h later); follicles were collected before and 4, 10, 20, 25, and 60h after GnRH.

Results: ET-2 exhibited a biphasic pattern of expression: it increased moderately at 4h, returned to basal levels, and dramatically rose again only in the early corpus luteum (CL; 60h post GnRH). However, ET-2 could not be detected in older CL. ET-2 in the young CL was identified in the luteinizing granulosa cells (GCs) but not in ECs. Similarly, in preovulatory follicles, higher ET-2 levels were observed in the GC layer than in the theca internal layer. Ample ECE-1 expression in GCs enabled the cells to secrete mature ET-2 peptide. We therefore studied next the in vitro regulation of ET-2 in GCs. In accordance with the in vivo data, cells incubated with LH exhibited a biphasic response, and a 2-fold increase was observed at 4 and 48h but not at 24h. Progesterone itself, or in conjunction with LH, did not further affect ET-2 levels. Before completion of angiogenesis, hypoxic conditions prevail in early CL. To examine whether hypoxia affects ET-2 expression, we incubated GCs with a hypoxia-mimicking agent, CoCL2. CoCL2 (50-200 uM) elevated ET-2 in GCs in a dose-dependent manner 3h after its addition, whereas the expression of the other ET peptide, ET-1, was inhibited. Interestingly, ETB and ETA levels followed a pattern similar to that of ET-2 and ET-1, respectively.

Conclusions: These studies suggest that the pattern of ovarian ET-2 expression is different in cows than in rats. Elevated ET-2 in the early bovine CL, triggered by LH surge and hypoxia, may possibly facilitate CL formation by promoting cell migration and differentiation.

Vitamin D attenuates the keratinocyte inflammatory response to TNF

Ms. Mor Miodovnik^{1,2} Dr. Amiram Ravid^{1,3} Prof. Ruth Koren^{1,2}

¹*Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel-Aviv University*

²*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University*

³*Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University*

Introduction: Natural and synthetic hormonally active derivatives of vitamin D are well known for their beneficial effects in the treatment of cutaneous inflammatory disorders, particularly in psoriasis. This effect is usually attributed to their anti-proliferative and pro-differentiative action on keratinocytes and to the inhibition of the activity of infiltrating immune cells. Since keratinocytes are known to play an important role in the induction and maintenance of cutaneous inflammation, we examined the notion that the attenuation of the inflammatory process by vitamin D is also due to interference with the pro-inflammatory action of the keratinocytes themselves.

Patients / Methods: Human HaCaT keratinocytes cultured in the absence of exogenous growth factors or active mediators were exposed to TNF to simulate an inflammatory challenge and their response was monitored by assessing mRNA levels of 3 key inflammatory genes using real-time PCR: the cytokine TNF, the chemokine IL-8 and the adhesion molecule ICAM-1.

Results: ICAM1 and IL-8 were induced rapidly peaking after 2h exposure to TNF and decreasing thereafter. mRNA levels of the same genes increased again from 8h onward to reach a plateau between 16h to 24h following treatment with TNF. TNF mRNA levels increased steadily between 2h and 24h after the inflammatory challenge. 24h pretreatment with calcitriol, the hormonal form of vitamin D, inhibited induction of IL-8 by ~ 70% but did not affect that of ICAM-1 or TNF 2h following exposure to TNF. On the other hand, calcitriol markedly inhibited the induction of all 3 pro-inflammatory genes 16h after the TNF challenge (inhibition of 85%, 70% and 50% of IL-8, ICAM-1 and TNF, respectively). Calcitriol inhibits the activation of Jun kinase (JNK) and p38-MAPK by TNF. Both these cascades are known to activate and induce AP-1 transcription factors. To find out whether the inhibitory action of vitamin D on gene induction could be mediated by JNK and p38-MAPK inhibition we examined the effect of the JNK inhibitor SP600125 and the p38-MAPK inhibitor SB203580. The combination of the two inhibitors fully reproduced the time and gene dependent modulatory effect of the hormone.

Conclusions: We conclude that vitamin D attenuates the active contribution of keratinocytes to cutaneous inflammation and that this modulatory effect can be explained by inhibition of the JNK and p38-MAPK cascades.

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MS-KIF18A Kinesin Is Regulated By Estrogen And Binds Estrogen Receptor

Ms. Margalit Zusev¹ Dr. Dafna Benayahu¹

¹*Department of Cellular and Developmental Biology, Sackler school of Medicine, Tel-Aviv University, Israel*

Introduction: MS-KIF18A is a kinesin-related protein composed of a motor domain, coiled-coil region and cargo binding domain. Kinesins facilitate trafficking of cell organelles, protein complexes or mRNA in cells.

Patients / Methods: MS-KIF18A expression and function was analyzed using MBA-15, a pre-osteogenic marrow stromal cell line. Distribution of the protein in various cellular compartments was demonstrated by cell imaging and fractionation. Potential posttranscriptional modification phosphorylation was suggested by bioinformatics and verified by calf intestinal alkaline phosphatase (CIAP) assay that dephosphorylates proteins or serum starvation of cell culture. Immunoprecipitation (IP) and western blot (WB) were used for the analysis of the proteins appearance. MS-KIF18A turnover was demonstrated using metabolic labeling of the cells with Met/Cis-S35. The expression of mRNA was studied using quantitative RT-PCR.

Results: We identified diverse forms of MS-KIF18A in cytoplasm and membrane/nucleus. Moreover, we have shown that MS-KIF18A undergoes phosphorylation and this posttranscriptional modification is likely regulates cellular distribution of the protein. Furthermore, we revealed that putative cargoes of MS-KIF18A are two Estrogen Receptor (ERalpha) isoforms, 66 and 46 kDa, which are expressed in MBA-15 cells. The MS-KIF18A binds ERalpha 46 kDa in estrogen dependent manner. In addition, we identified that the cytoplasmatic form of MS-KIF18A is prevalent and mainly binds ERalpha 66 kDa. The cells' challenge with 17beta-E2 resulted with an increase in MS-KIF18A mRNA and protein expression. We detected a faster turnover of MS-KIF18A in cells treated with 17beta-E2 then in untreated once.

Conclusions: 17beta-E2 triggers transcription, translation and degradation of MS-KIF18A, which in its turn bind ERalpha in cytoplasm in estrogen dependent manner. This study contributes to the understanding of MS-KIF18A function and suggests its role in regulation of ERalpha localization in mesenchymal stromal cells.

Effects of androgens and estrogens on 12- and 15-Lipoxygenase Expression and Activity in Cultured Human Female Derived Osteoblasts

Prof. Dalia Somjen¹ Dr. Sara Katzburg¹ Ms. Esther Knoll¹ Ms. Orly Sharon¹
Dr. Rona Limor¹ Dr. David Hendel² Prof. Naftali Stern¹

¹*Institute of Endocrinology, Metabolism and Hypertension, Tel- Aviv Sourasky Medical Center, and The Sackler Faculty of Medicine, Tel- Aviv University, Tel- Aviv*

²*Department of Orthopedic Surgery, Sharei- Zedek Medical Center, Jerusalem,*

Introduction: Recent observations that 12/15 lipoxygenase (LO) KO mice show higher bone density and that several polymorphisms in either the platelet type 12LO or 15LO genes are linked to bone density in human subjects have raised interest in the potential role of lipoxygenase (LO) enzymes in bone physiology. Since gonadal steroids are established modulators of bone formation and resorption, we examined the effects of androgens and estrogens on LO in primary cultures of human-osteoblast-like cells (hObs) harvested during orthopedic surgery.

Patients / Methods: Cells from pre- and post- menopausal females were grown as described before and were analysed for the expression of platelet type- 12LO and 15LO mRNA (both type I and type II) and release both 12- and 15- hydroxyeicosatetraenoic acid (HETE) after hormonal treatments.

Results: Cells from pre- and post- menopausal females express both platelet type- 12LO and 15LO mRNA (both type I and type II) and release both 12- and 15- hydroxyeicosatetraenoic acid (HETE). Dihydrotestosterone (DHT), but not estradiol17b (at 300 and 30nM respectively) induced within 10 min a marked increase in the production of both 12- and 15-HETE and within 3 days in the mRNA expression of 12- and 15-LO in cells harvested from either pre- or post- menopausal women. While estradiol17b per se was inactive in terms of HETE generation, the phytoestrogen biochanin A (BA; 3mM) stimulated both 12- and 15-HETE production. BA also upregulated 12- and 15- LO mRNA expression, whereas estradiol17b had a modest effect on 12-LO mRNA expression only.

	12HETE	12LOmRNA	15HETE	15LOmRNA
DHT	25+11% *	227+19%***	320+15%***	110+10%**
	40+12%*	127+25%**	580+20%***	75+23%*
	33+11%*	177+22%**	450+35%***	93+17%**
BA	347+16%***	99+13%**	170+30%**	33+5%*
	156+12%**	197+3%**	107+30%*	168+4%**
	251+14%***	148+8%**	138+30%**	100+4%**
E2	-25+19%ns	54+17%*	15+12%	-40+8%*
	-20+20%ns	52+17%*	2+15%	36+5%*
	-23+20% ns	53+17%*	8+13%	-2+6%

*p<0.01; **p<0.05, ***p<0.001

Conclusions: Collectively these results indicate that human female osteoblast- like cells express both 12- and 15-LO, whose activity and expression levels are turned on by androgens and not by native estrogens. In contrast, BA, a phytoestrogen presumed to operated through classical estrogen receptors, affects bone cell LO in a manner more similar to that of native androgen than estrogens. Because HETES regulate bone cell proliferation, the functional implications of these findings await further clarification.

Clinical characteristics of patients treated with Teriparatide after long term antiresorptives treatment in a single tertiary referral metabolic clinic

Dr. Gloria Tsvetov¹ Dr. Carlos Benbassat^{1,2} Dr. Ilana Slozki¹ Dr. Varda Eshed³

¹*Endocrine Institute, Rabin Medical Center, Petah Tikva*

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

³*Medical Department, Eli Lilly, Israel*

Introduction: Teriparatide [rhPTH(1-34)] has been shown to increase bone formation, and bone mass and decrease the risk of fractures in postmenopausal women and men with osteoporosis. In most clinical studies, teriparatide was administered to treatment-naive patients. The aim of our study was to characterize and observe response to teriparatide treatment, in patients with osteoporosis previously treated with long term antiresorptives drugs, in a real life setting of our tertiary referral metabolic clinic.

Patients / Methods: This was a retrospective observational study. Data concerning baseline characteristics and response to treatment was collected from medical charts of consecutive patients who started treatment with teriparatide in our clinic between the years 2004-2006. All patients were treated with calcium and vitamin D3-25OH supplements along with the regular dose of teriparatide 20mcg once daily. All patients had normal levels of calcium, parathyroid hormone (PTH) and vitamin D3-25OH and normal renal and liver function.

Results: During the years 2004-2006, 35 osteoporotic women and one man (mean age 71.3+9.22) started treatment with teriparatide. Mean treatment period was 13.35+9.22 months. At baseline 75% of patients had previous fractures. All patients except two received long term previous anti-resorptives treatment (mean treatment period 6.8+3 years), mostly bisphosphonates. The most common indication for starting teriparatide was recent fractures (64%). Other indications were: deterioration of BMD (28%) and very low bone mass (8%). Follow-up BMD tests were done after a mean period of 13 months. Statistically significant elevations in BMD were observed in the lumbar spine (mean 7%+7.3, p=0.001, total hip (4%+6, p=0.028) and femoral neck (mean 2.5%+5, p=0.055). A statistically significant elevation was also observed in ALP levels, which rose by a mean of 35%+31 (p<0.001) after the first month of treatment. Three patients (8.3%) suffered new fractures during treatment. Seven patients (20%) reported an improvement in back pain accompanied by a decrease in the demand for analgesic drugs. The treatment was well tolerated. Transient hypercalcemia (<12 mg/dl) and hypercalciuria were detected in 7 patient (20%). None of the patients discontinued treatment due to problems in injecting the drug or adverse events.

Conclusions: Teriparatide was administered to patients suffering from severe osteoporosis, most of them treated previously for long term with antiresorptives drugs. The treatment resulted in significant elevations of BMD and ALP and reduction of back pain and was accompanied by a small number of fractures. Treatment was well tolerated.

The involvement of Estrogen in accelerated Osteogenesis in skeletal growth center: A proposed mechanism

Ms. Marina Gurman¹ Prof. Moshe Phillip² **Dr. Gila Maor**¹

¹*Department of Anatomy and Cell Biology, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa*

²*and Institute of Endocrinology and Diabetes, Schneider Children's Medical Center, Petach Tikva*

Introduction: Major skeletal changes occurring at puberty like growth spurt and closure of epiphyseal growth plates (EGP) leading to growth cessation are modulated by gonadal steroids. Estrogen (E2) is implicated with ossification of EGP and growth arrest in both genders. However, in other tissue including bone E2 is anabolic. It is not yet understood what mediates the catabolic activity of E2 in EGP. E2 exerts its biological effects through two receptors isotypes: ERalpha and ERbeta which act at both nuclear and membrane levels. The current study is aimed at elucidating the mechanisms that mediate E2-induced accelerated osteogenesis.

Patients / Methods: Method: Two experimental models of endochondral ossification were used; a murine derived Mandibular condyle (MC) organ culture (MCOC), and MC derived primary tissue culture (MCDC). The ER isotypes present in the cartilage cells were determined using immunoblotting (IB) and immunohistochemistry (IHC). The cellular localization of ER was determined using immunoprecipitation (IP). The biological activity of the membrane receptor was determined using the cell impermeable derivative E2BSA. The effects on chondrogenesis and osteogenesis were estimated by following proliferation rate, proteoglycans expression and apoptosis factors. The involvement of ERs in cellular pathways was evaluated by following pAKT, pERK and pp38 expression, using ER specific modulators and inhibitors.

Results: We have shown that both ERalpha and ERbeta are present in cartilage cells of growth centers, and that these receptors are distributed in both membrane and cytoplasmic fractions. Using organ and tissue culture models we showed that E2, opposed to E2BSA, accelerates maturation of skeletal growth center by interfering in proliferation and differentiation rates of chondrocytes-causing uncoupling between the two. Furthermore, E2 exerts its effects mainly through ERα which appears to be implicated with E2 anti anabolic effect. These E2 activities are transduced specifically via p38 MAPK pathway, although ERK pathway seems also to take part in mediating E2 - induced EGP maturation.

Conclusions: We have shown that estrogen directly modulates skeletal growth rate causing its final closure. These catabolic effects of E2 are mediated mainly by cytoplasmic receptor-most likely ERβ. These growth arrest activities are mediated via the p38 stress MAPK leading to enhanced chondrocytes apoptosis accompanied by accelerated ossification. These observations might also enlighten the mechanisms responsible for the versatile activities of estrogen in various organs.

Vitamin D Deficiency in Oncologic Patients - an Ignored and Potentially Life Threatening Condition, the Role of Osteoblastic Activity in Hypocalcemia

Prof. Sophia Ish-Shalom^{1,4} Dr. Shira, Shira Felder² Dr. Elena Segal¹ Mrs. Hedva Yoffe- Sheinman² Dr. Mira Volner² Dr. Eliahu, Eliahu Gez² Mrs. Batia Raz³ Miss Zila Shen- Or³ Dr. Nissim Haim³

¹*Metabolic Bone Diseases Unit, Ramabam Health Care Campus, Haifa*

²*Department of Oncology, Ramabam Health Care Campus, Haifa*

³*Endocrine Laboratory, Ramabam Health Care Campus, Haifa*

⁴*The Bruce Rappaport Faculty of Medicine, Technion -Israel Institute of Technology, Haifa*

Introduction: The aim of this work was to assess vitamin D status and bone turnover in oncologic patients and their impact on the risk of hypocalcemia.

Monthly administration of bisphosphonates to oncologic patients with skeletal involvement, decreases bone turnover and in combination with poor vitamin D status may lead to life threatening hypocalcemia.

Recently three patients on intravenous bisphosphonate treatment for metastatic bone disease, aged 53.33±17.7 were hospitalized for severe symptomatic hypocalcemia of 6.5-7 mg/dl, 25(OH)D3 levels were 2.4-12 ng/ml, serum PTH - 73-275 pg/l.(normal 11-65)

We were informed that routine or sporadic (based on medical history) evaluation of vitamin D status and vitamin D supplementation is not included in the national or international guidelines for bisphosphonate treatment for metastatic bone diseases.

Patients / Methods: Following these findings, after IRB approval, we have assessed serum calcium, phosphate, albumin, 25(OH)D3 and plasma PTH, procollagen type1 nitrogenous propeptide (P1NP), C-terminal telopeptide of type 1 collagen (CTX) in 22 consecutive patients with metastatic bone disease, aged 58.7±11.6 range 42-86, that were treated monthly with intravenous bisphosphonates (pamidronate or zoledronate).

Results: Serum albumin corrected calcium was 9.46 mg/dl ±0.9, range 6.7-11.2; 25(OH)D3 serum level was 12.9 ±4.7 : ng/ml, vitamin D deficiency < 10 ng/ml: 5 pt; vitamin D insufficiency 10-15 ng/ml: 8 pt, vitamin D inadequacy <30 ng/ml: 8pt (<20.3). Plasma P1NP was 149.7, 251.3, markedly above premenopausal range of 15.13-58.59ng/ml. Albumin corrected calcium strongly negatively correlated with P1NP (R(-) 0.8) ;P< 0.0001) and with serum alkaline phosphatase (R- 0.72, P< 0.0001). Plasma CTX level was 0.27 ±0.18 (within premenopausal range of 0.299-0.14: ng/ml) and has not correlated with albumin corrected calcium.

Conclusions: Inadequate 25(OH)D3 serum levels are common in oncologic patients; they are prone to hypocalcemia due to bisphosphonate treatment and absence of supplementation with calcium and vitamin D, especially in presence of increased osteoblastic activity.

Hypocalcemia and vitamin D deficiency are a preventable conditions; evaluation of vitamin D status and adequate calcium and vitamin D supplementation should be a part of management of oncologic patients.

Evaluation of the Hypothalamic-Pituitary-Adrenal Axis and Metabolic Profile of Women with Polycystic Ovary Syndrome

Dr. Elvira Chen¹ Dr. Caroline Apovian² Dr. Suzy Kovatz³ Dr. Ghazy Ganem⁴
Dr. Pnina Rotman-Piekelnny⁵ Dr. Michael Pomeranz⁶ Mr. Eliahu Weiss⁷
Prof. Menachem Shapiro⁸ Prof. Louis Shenkman³ **Dr. Rosane Ness-Abramof**⁸

¹Department of Medicine B, Sapir Medical Center, Kfar Saba

²Section of Endocrinology, Diabetes, and Nutrition, Boston Medical Center, Boston

³Department of Medicine C, Sapir Medical Center, Kfar Saba

⁴Kupat Holim Dgani, Hadara

⁵Department of Medicine E, Sapir Medical Center, Kfar Saba

⁶Department of Obstetric and Gynecology, Sapir Medical Center, Kfar Saba

⁷Endocrine Laboratory, Sapir Medical Center, Kfar Saba

⁸Endocrine Unit

Introduction: Polycystic Ovary Syndrome (PCOS) is a common endocrinopathy, occurring in 5-7% of women in the reproductive age. The hallmark of the syndrome is menstrual irregularity (oligomenorrhea or amenorrhea) and hyperandrogenism. In women with PCOS there is a cluster of metabolic abnormalities that increase the risk for cardiovascular disease. The combination of insulin resistance and hyperandrogenism raises the possibility of hypercortisolism as a pathogenetic factor. Previous studies have found that up to 50% of women with PCOS have increased urinary free cortisol. We therefore decided to evaluate the metabolic profile of women with PCOS in order to assess if they are hypercortisolemic or have abnormal cortisol suppression.

Patients / Methods: Ten women with PCOS and 10 control women were recruited. Anthropometric measurements (BMI and waist circumference), blood pressure and fasting glucose, insulin, c-peptide, cholesterol, triglycerides, HDL, C-reactive protein (CRP), testosterone, free testosterone and DHEAS were measured. The HPA axis was evaluated by measuring morning ACTH and cortisol, urinary free cortisol and 1 mg dexamethasone suppression test (ODST).

Results: Mean age of the women with PCOS was 26 ± 6 years and of the control group 32 ± 5 years ($P=0.023$). Patients with PCOS had higher BMI (35.9 kg/m^2 vs 23.7 kg/m^2 , $P=0.001$), waist circumference (99.6 cm vs 77.8 cm , $P=0.002$) and systolic blood pressure (117 mmHg vs 107 mmHg , $P=0.043$). Glucose, insulin, total cholesterol, HDL-cholesterol and triglycerides were similar in both groups, while LDL-cholesterol was higher in women with PCOS. CRP levels were higher in women with PCOS (0.75 mg/dl vs 0.13 mg/dl , $P=0.009$). Testosterone levels were higher (0.54 vs 0.37 , $P=0.014$) and sex hormone binding globulin (SHBG) lower (25.1 vs 63.7 , $P=0.02$) in the PCOS group. ACTH was higher in women with PCOS (24.9 vs 15.2 , $P=0.41$) while morning cortisol and 24 hour urinary free cortisol were similar in both groups ($12.5 \text{ } \mu\text{g/dl}$ vs $11.3 \text{ } \mu\text{g/dl}$; $61.5 \text{ } \mu\text{g}/24 \text{ h}$ vs $68.2 \text{ } \mu\text{g}/24 \text{ h}$ respectively). Cortisol levels after 1 mg ODST suppressed to less than $1.8 \text{ } \mu\text{g/dl}$ in all patients.

Conclusions: Women with PCOS have higher baseline ACTH levels but not other signs of HPA hyperactivity. Urinary free cortisol and dexamethasone suppression is normal in obese women with PCOS and did not correlate with markers of insulin resistance and the metabolic syndrome.

Disease Control And Sleep/Activity Quality By Morning Or Evening Replacement Therapy In Congenital Adrenal Hyperplasia

Alina German¹ Suhir, Suhir Suraiya³ Yardena Tenenbaum-Rakover^{4,5} Ilana Koren¹ Giora Pillar^{3,5} Ze'ev, Ze'ev Hochberg^{2,5}

¹Clalit HMO, Haifa

²Pediatric Endocrinology Unit, Meyer Children's Hospital, Haifa

³Sleep Laboratory, Rambam Medical Center, Haifa

⁴Pediatric Endocrine Unit, Ha'Emek Medical center, Afula

⁵Technion Faculty of Medicine, Haifa

Introduction: Background: In the treatment of congenital adrenal hyperplasia (CAH), some attempt to improve early morning ACTH suppression and endocrine control by a high bedtime hydrocortisone (HC) dose, while others imitate cortisol diurnal variation, expecting the axis to play a role in sleep quality and daytime activity.

Hypotheses: Higher morning HC dose simulates physiological pattern of HPA function and allows better sleep and activity pattern. Higher night dose effectively suppresses early morning ACTH peak levels and improves disease control.

Patients / Methods: Design: A cross-over study of 15 CAH (classical form) patients, age 7-18 y. The patients were treated by their regular HC dose of 11-17 mg/m², and were randomized to receive for 2 weeks either higher morning or evening dose - 50%, with 25% at noon and 25% at evening or morning, respectively, followed by 2 weeks of the reciprocal regimen. During the second week of each treatment schedule sleep and daytime activity were monitored by a 7-day actigraph. Serum levels of 17OHP, testosterone, DHEA-S, and androstendione were measured at 8 am of the last day of each treatment regimen.

Results: Results: No differences were found in total sleep time between the morning and evening regimens (463 ± 50 vs. 466 ± 52 min). The number of arousals did not differ (4.7 ± 2.8 vs. 4.4 ± 1.7). No differences were found in sleep efficiency (86 ± 8 vs. 87 ± 6 %), daytime activity index (60 ± 12 vs. 59 ± 8) and in basal morning 17OHP levels (22.7 ± 25.1 vs. 23.8 ± 27.6 nmol/l), testosterone levels (2.8 ± 5.1 vs. 2.3 ± 3.0 nmol/l), DHEAS levels (2.4 ± 4.6 vs. 1.8 ± 3.6 nmol/l) and androstendione levels (2.2 ± 1.6 vs. 4.2 ± 4.3 pmol/l).

Conclusions: Conclusions: Since no differences were observed in disease control, sleep quality or daytime activity between either treatment schedules, we recommend the higher morning HC dose regimen because it simulates the physiological diurnal rhythms of the HPA axis.

MUTATIONS IN EPITHELIAL SODIUM CHANNEL (ENAC) BETA SUBUNIT GENE RESPONSIBLE FOR MULTI-SYSTEM PSEUDOHYPOALDOSTERONISM IN ASHKENAZI FAMILIES

Mr. Oded Edelheit^{1,2} Prof. Israel Hanukoglu¹ Mr. Matanel Tefilin¹ Dr. David Gillis³ Dr. David H. Zangen³ Dr. Gheona Altarescu⁴ Prof. Aaron Hanukoglu^{2,5}

¹*Dept. of Molecular Biology, College of Judea and Samaria, Ariel*

²*Tel-Aviv University, Sackler Medical School*

³*Dept. of Pediatrics, Hadassah-Hebrew University Medical Center, Jerusalem*

⁴*Genetics Unit, Shaare Zedek Medical Center*

⁵*Div. of Pediatric Endocrinology, E. Wolfson Hospital, Holon*

Introduction: Multi-system pseudohypoaldosteronism (PHA) is a syndrome of aldosterone unresponsiveness that leads to severe salt-wasting in early infancy. Previously we showed that PHA results from mutations in genes encoding Epithelial sodium channels (ENaC) subunits. ENaC is composed of three related subunits (alpha, beta and gamma) encoded by three genes located on chromosomes 12 and 16. These genes are expressed in aldosterone-responsive epithelial cells lining the distal renal tubule, colon, respiratory airways and exocrine glands such as salivary and sweat glands and play a major role in the regulation of electrolyte balance, blood volume and pressure.

Patients / Methods: A female infant born as the second sibling of non-consanguineous Ashkenazi Jewish parents presented with extreme lethargy at age 8 days with one-day history of poor feeding. On admission sodium=132 mmol/l and potassium=9.2 mmol/l. Trans-tubular potassium gradient was 2.4, indicating very low renal potassium excretion. Sweat Cl =137 mmol/l (normal 15 ng/ml/hour (normal 2800 pmol/l values were consistent with a diagnosis of PHA. At the age of one year she still requires large quantities of NaCl, sodium bicarbonate, kayexalate therapy and low K diet. Apart from electrolyte disturbances she also suffers from failure to thrive, chronic nasal discharge, seborrhea like skin eruption and recurrent respiratory infections. To examine whether PHA is caused by mutations in ENaC genes, we sequenced alpha, beta and gamma ENaC genes.

Results: We found two heterozygous mutations, an insertion and a deletion, in exons 4 and 6 of the beta ENaC gene. Both mutations cause frameshifts in the coding sequence. Thus, the affected case was clearly a compound heterozygote. To identify the origin of the mutations we sequenced the ENaC genes of both parents and confirmed that they are heterozygote carriers of different mutations. We had previously identified PHA only in isolated cases in Iranian Jewish patients in Israel and in diverse ethnic groups from around the world.

Conclusions: This is the first case of PHA in the Ashkenazi community in Israel. In the USA, a single case of PHA has been reported for an Ashkenazi Jewish patient. If multi-system PHA is undetected during the first week of life it may lead to neonatal death. Early detection and appropriate therapy (life long high salt diet) may lead close to normal lives. In view of the compound heterozygote mutations seen here and in the USA there may be a need for screening among the Ashkenazi population to identify carriers. Families with carrier members should be provided genetic counseling and prenatal diagnosis.

Absence of the PPARalpha (PPARa) Gene Abolishes Hypertension, and Attenuates Atherosclerosis Through Downregulation of the Renin-Angiotensin-Aldosterone System in the Tsukuba Hypertensive Mouse (THM)

Dr. Karen M. Tordjman¹ Prof. Clay F. Semenkovich² Dr. Trey Coleman² Dr. Ety Osher¹ Dr. Michal Vechoropoulos¹ Prof. Nafatli Stern¹

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

²*Division of Endocrinology, Metabolism & Lipid Research, Washington University School of Medicine, St. Louis, Missouri, USA*

Introduction: Among its pleiotropic effects, PPARa is also believed to protect against atherosclerosis by directly acting at the vessel wall. In the Tsukuba Hypertensive Mouse (THM), transgenic expression and activation of the human renin-angiotensin system (RAS), results in hypertension and atherosclerosis due to high levels of angiotensin II (AII) and aldosterone.

We previously reported that making these mice deficient in PPARa (THM/PPARKO) totally abolished the hypertension. We further investigated the effect of PPARa deficiency on the functional expression of the RAS, and its impact on the extent of atherosclerosis.

Patients / Methods: After feeding the mice an atherogenic diet for 12 weeks, blood chemistry and hormonal determinations were made, and blood pressure was assessed. Peritoneal macrophages were harvested to examine foam cell formation, and hearts were retrieved for quantitation of atherosclerosis at the aortic sinus.

Results: Despite being heavier than THM, THM/PPARKO mice remained normotensive throughout the experiment. After 12 weeks, lipid levels were identical in both models, however THM/PPARKO had lower fasting blood glucose levels (103±19 mg/dl vs. 146±18 mg/dl in THM, P=0.001), and better intraperitoneal glucose tolerance. The absence of PPARa resulted in decreased plasma human active renin, 3525±128 mU/L vs 1909±755 mU/L, P=0.01. Primary cultures of aortic smooth muscles cells from THM/PPARKO showed markedly suppressed human renin secretion. Further, the expression of the AII type 1 receptor in the aorta of these animals was significantly decreased. Not surprisingly, THM/PPARKO mice had reduced aldosterone levels 517±66 pg/ml, compared to the elevated levels measured in the THM animals, 1209±215 pg/ml, P=0.02.

The extent of atherosclerosis at the aortic sinus was decreased by over 80% in the THM/PPARKO animals compared to THM. In addition, the 92% decrease in the foam cell formation from peritoneal macrophages in THM/PPARKO suggested that the atherogenic process was also affected independently of blood pressure, possibly as a result of reduced oxidative stress due to lower prevailing AII levels.

Conclusions: In conclusion, absence of PPARa in THM not only abolished the activated RAS-induced hypertension, but also greatly attenuated the concomitant atherosclerosis. These effects were accompanied by evidence for functional downregulation of the entire RAS. We therefore suggest that under certain circumstances PPARa may lead to further activation of the RAS and could be detrimental.

Inhibition of 12-lipoxygenase by baicalein reduced in vitro cell proliferation and induced apoptosis in two human primary adrenocortical carcinoma

Dr. Anat Jaffe¹ Mrs. Sholamit Karby¹ Mrs. Keren Cohen¹ Dr. Shalom Bar-AMI¹

¹*Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera*

Introduction: Adrenal cortex cancer is a highly malignant tumor for which no effective medical treatment currently exists. In previous study, we demonstrated expression of 12-Lipoxygenase platelets type [12LOX-p] and 15-Lipoxygenase type 1 [15LOX-1] in H295R, a human adrenocortical carcinoma (ACC) cell line. Moreover, we showed that 12LOX-p has a key role in the prevention of apoptotic cell death.

Baicalein is a flavonoid extracted from the root of *Scutellaria baicalensis* Georgi, a medicinal plant traditionally used in Oriental medicine. Inhibition of 12LOX by Baicalein exerts pro-apoptotic effects in; myeloma RPMI8226, leukemia cells, neuroblastoma, fibrosarcoma, human renal cell carcinoma and H295R. In the present work, we have studied the in-vitro effects of Baicalein on two primary ACC cells that have been obtained from our two patients.

Patients / Methods: The patients: A 29-year-old woman presented with rapidly progressive Cushing symptoms [A] and a 68-year-old woman presented with abdominal pain, stage IV disease and laboratory Cushing [B]. After removal of the surgical specimens, 1.5mL of tissue was aseptically excised from the tumor, minced, rinsed and chopped up into pieces, which were plated as mini explants on 6cm wells. The mini explants grow polygonal cells with big nuclei, prominent nucleoli and many peri-nuclei cytoplasmic granules.

The effects of Baicalein on cell proliferation were tested by cell counting. Apoptotic changes were examined under fluorescence microscopy using double staining Annexin V and propidium iodide (PI). Expression of 12LOX-p and 15LOX-1 was determined by RT-PCR from total RNA collected on passages 4 to 6.

Results: We have established two primary human ACC cells for 12 passages. Addition of 10⁻⁵M Baicalein for 24, 48 and 72 hours reduced cell density in A+B ACC cells. The reduced cell density was due to a significantly increase in apoptosis. The results are presented in percent of apoptosis for control and Baicalein respectively [$* P < 0.05$ Baicalein vs. control]. At 24h; 10.4±3.6 vs. 19.5±4.8* for A and 4.5±.9 vs. 12.2±5.3 for B. At 48h; 14±2.5 vs. 25.5±1.2* in A and 1.3±.3 vs. 15.8±6.5* in B. At 72 h; 14.1±3.1 vs. 22±2.8* for A and 2.4±.7 vs. 24.7±.3* in B, n=3.

Using RT-PCR we have detected the gene expression of 12LOX-p and 15LOX-1 in A- ACC cells. However, in B- ACC cells, only 12LOX-p expression was detected.

Conclusions: The apoptosis-inducing properties of Baicalein in H295R are relevant also for the two primary human ACC cells. The effect of Baicalein is probably occurring through the 12LOX-p inhibition, which is expressed in both ACC cells.

This traditionally used oriental medicine should be further examined in in-vivo models.

Role of PKC in GnRH activation of Extracellular Signal-Regulated Kinase (ERK) and Jun N-Terminal Kinase (JNK)

Mrs. Masha Dobkin-Bekman¹ Prof. Rony Seger² Prof. Zvi Naor¹

¹*Department of Biochemistry, Tel Aviv University, Ramat Aviv 69978, Israel*

²*Department of Biological Regulation, The Weizmann Institute of Science Rehovot 76100, Israel*

Introduction: Hypothalamic gonadotropin releasing hormone (GnRH) stimulates the synthesis and release of the glycoprotein hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH) from pituitary gonadotropes. Signaling of GnRH in pituitary gonadotropes include activation of phospholipases C beta, enhanced phosphoinositide turnover, sequential activation of phospholipases D and PLA2, Ca²⁺ mobilization and influx, activation of PKC followed by stimulation of the MAPK cascades ERK, JNK and p38, culminating in gonadotropin release and synthesis.

Patients / Methods: The role of PKC isoforms in GnRH-stimulated MAPK was examined in the gonadotrope cell lines alphaT3 and LbetaT2.

Results: Incubation of the cells with GnRH resulted in a protracted activation of ERK1/2 and a slower and transient activation of JNK1/2. The use of GFP-PKCs constructs revealed that GnRH induces a rapid translocation of PKCalpha and PKCbetaII to the membrane, followed by redistribution to the cytosol, while PKCdelta and PKCepsilon localize to the cytoplasm and Golgi, followed by rapid redistribution (PKCdelta) to the perinuclear zone and a prolonged translocation (PKCepsilon) to the membrane. The pan PKC isoforms inhibitor, GF109203X nearly abolished GnRH stimulation of ERK1/2, but only reduced JNK1/2 activation by 50%. The conventional PKC (cPKC) inhibitor, Go 6976, reduced GnRH stimulation of ERK1, but markedly inhibited ERK2 activation and abolished GnRH stimulation of JNK1/2. The selective PKCdelta inhibitor, rottlerin, markedly inhibited GnRH stimulation of ERK1/2 with lesser effect on JNK1/2. GF109203X abolished PMA-stimulation of ERK1/2 and JNK1/2. As with GnRH, Go 6976, was a more potent inhibitor of PMA stimulation of ERK2 (60%) vs. ERK1 (20%), but JNK1/2 activation was abolished. Unlike GnRH, rottlerin reduced significantly PMA signaling to JNK1/2. Co-expression of GFP epitope-tagged ERK2 and dominant-negative plasmids of PKCs revealed that PKCbetaII and PKCdelta mediate ERK2 activation by GnRH and PMA. A cPKC isoform is involved in both GnRH and TPA-stimulation of JNK1/2, while PKCdelta participates in PMA but not in GnRH signaling to JNK1/2.

Conclusions: Comparing the alphaT3-1 to the more mature LbetaT-2 gonadotropes revealed that the role of PKC in GnRH to ERK signaling diminishes with maturation of pituitary gonadotropes and the maturation has produced a switch in the relative sensitivity of ERK1 vs. ERK2 to the PKC isoforms inhibitors. The above data suggest that GnRH and PMA activate ERK and JNK by common and separate PKC isoforms and indicate for the first time differential role of PKC in ERK1 vs. ERK2 activation by a GPCR.

Placental Expression of PKBalpha/Akt1 Improves Survival but does not Rescue PKBalpha/Akt1- deficient Embryos from Intrauterine Growth Retardation

Ms. Vicki Plaks¹ Ms. Elina Berkovitz² Dr. Tamara Berkutzki² Dr. Rebecca Haffner² Dr. Alon Harmelin² Dr. Brian A. Hemmings³ Dr. Nava Dekel¹ Dr. Michal Neeman¹

¹*Biological Regulation, The Weizmann Institute of Science, Rehovot, 76100, Israel*

²*Veterinary Resources, The Weizmann Institute of Science, Rehovot, 76100, Israel*

³*Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland*

Introduction: The importance of the placental circulation has long been recognized and is exemplified by the correlation of placental size and blood flow with fetal weight during normal pregnancies in many mammalian species. Complications of pregnancy such as preeclampsia and intrauterine growth restriction are strongly associated with impaired placental development and function. The rates of placental blood flow are dependent on placental vascularization, and placental angiogenesis is therefore critical for the successful development of a viable, healthy offspring. PKB/Akt is known to act downstream of VEGF, a major angiogenic factor. In addition to angiogenesis, PKB regulates many other cellular and physiological processes as glucose metabolism, transcription, cell cycle regulation, survival and inflammation. PKBalpha was previously found to be widely expressed in placenta. Placentas of PKBalpha null fetuses displayed decreased size and impaired vascularization. The PKBalpha null fetuses exhibited reduced weight accompanied by reduced survival. In this work the focus was put on differentiating between the role of PKBalpha in placentation from its impact on the overall fetal wellbeing.

Patients / Methods: In order to evaluate the role of placental vascular function in PKBalpha/Akt1 null intrauterine growth retardation, we substituted the embryos with a PKBalpha-expressing tetraploid placenta. The inner cell mass of PKBalpha null and heterozygous blastocysts were aggregated with the tetraploid embryos and transplanted into ICR pseudopregnant mice. Non-invasive imaging using macromolecular dynamic contrast enhanced MRI was applied for evaluating placental vascular function of PKBalpha expressing and null placentas.

Results: Complementing PKBalpha deficient embryos with tetraploid PKBalpha expressing placenta, resulted in significantly higher in-utero survival of the embryos, but was not sufficient for prevention of intrauterine growth retardation. MRI allowed quantification of maternal placenta blood volume that was found to be significantly reduced in PKBalpha null placentas. Tetraploid placentas were shown to have similar vascular characteristics as normal, healthy placentas.

Conclusions: Complementation experiments using PKBalpha expressing tetraploid placenta allowed differentiation between the roles of placental versus fetal PKBalpha: While placental PKBalpha regulates fetal survival in-utero, fetal PKBalpha regulates fetal growth.

Activin as a Possible Anti-Apoptotic Agent: Relevance for Neurodegenerative Diseases

Mrs. Lana Kupersmidt Amit T, Orith Bar-Am, Youdim M.B.H, Blumenfeld Z.

The Rappaport Faculty of Medicine, Technion, Israel Institute of Technology,

Introduction: Activin is a member of the transforming growth factor (TGF) -beta superfamily which comprises a growing list of multifunctional proteins that function as modulators of cell proliferation, differentiation, hormone secretion and neuronal survival. In the CNS, Activin is involved in development, being constitutively produced and widely distributed in the embryonic and adult peripheral and central nervous system.

Patients / Methods: The aim of the present study was to gain new insight into the mechanism(s) by which Activin-A and -B may regulate neuronal neuroprotection. Here, we show that Activin-A or -B (10-25 ng/ml) significantly reduced cell death, as induced either by serum deprivation, the parkinsonism-inducing neurotoxin, 6-hydroxydopamine (6-OHDA) or the peroxynitrite donor, SIN-1 in human SH-SY5Y neuroblastoma cells. Neuronal cell injury was evaluated by a colorimetric assay for mitochondrial function, using MTT test. Transient transfections with betaA-, betaB- pcDNA3.0 plasmids or empty pcDNA3.0 vector were performed, as well as western immunoblot analyses.

Results: We found that transient transfection with the Activin-beta-A and beta-B vector in SH-SY5Y, rat pheochromocytoma PC12, and human U-87-MG glioma cells resulted in the protection of cells from apoptosis, compared with respective cells transfected with a control plasmid. The neuroprotective effect involved inhibition of the cleavage and activation of pro-Caspase-3 and poly (ADP-ribose) polymerase (PARP), induction of anti-apoptotic proteins, Bcl-2 and BclL and reduction in the pro-apoptotic protein, Bad. These effects were associated with up-regulation of tyrosine hydroxylase expression. Follistatin inhibited the neuroprotective effects observed in SH-SY5Y cells, transiently transfected with beta-A- or beta-B-pcDNA3.0, indicating the specificity of Activin A and B in neuroprotection.

Immunoneutralization of SH-SY5Y/ beta-A -pcDNA3.0 or SH-SY5Y/ beta-B -pcDNA3.0 with the respective antibodies, anti-Activin A and anti-Activin B, also significantly inhibited neuroprotection against serum deprivation. Similar results were obtained with rFollistatin and anti-Activin A and B antibodies in Activin beta-A- or beta-B-overexpressing PC12 cells. No effect was observed in cells transfected with the empty vector.

Conclusions: In conclusion, the present study indicates that both Activin A and B possess neuroprotective activity associated with regulation of Bcl-2 family proteins. This may be of clinical importance, since the application of exogenous Activin A or B or stimulation of Activin A or B expression might have positive impact on aging and neurodegenerative diseases or acute neural trauma to retard the accelerated rate of neuronal degeneration.

Vitamin D Induces COX-2 In Human Keratinocytes

Ms. Efrat Buchner^{1,2} Prof. Ruth, Ruth Koren^{1,2} Dr. Amiram Ravid^{1,3}

¹*Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel-Aviv University*

²*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University*

³*Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University*

Introduction: Exposure of the epidermis to xenobiotics, allergens, invasive microorganisms as well as its injury or irradiation, elicits an inflammatory response in the keratinocytes. Prostaglandins take part both in mediating inflammation and in its resolution. One of the key enzymes in prostaglandin production is cyclooxygenase, which has a constitutive form (COX-1) and an inducible form (COX-2). Calcitriol, the hormonally active metabolite of vitamin D, was shown to modulate the cutaneous response to stress. Our aim was to examine the effect of calcitriol, alone or in the presence of inflammatory mediators, on COX levels in human keratinocytes

Patients / Methods: Our experimental model was the immortalized non-tumorigenic HaCaT keratinocytes that can proliferate autonomously and were cultured in the absence of exogenous growth factors or active mediators. Inflammation was simulated by exposure to TNF α . Protein levels were measured by western blotting. In situ COX activity was measured by assaying PGE2 levels produced in culture in the presence of exogenous arachidonic acid. mRNA levels were measured using TaqMan-based real-time PCR.

Results: 24h incubation with calcitriol increased COX-2 protein levels in HaCaT cells (4 fold increase at 100nM). This effect was dose dependent (already significant at 10 nM). Under identical conditions the level of COX-1 was not affected. Exposure to TNF α (20 ng/ml, 24h) resulted in a 2 fold increase in COX-2 levels. The effects of the two agents were additive. The biological significance of these results was demonstrated by the generation of PGE2 that was in correlation with the cellular COX-2 levels. COX-2 mRNA increased within 1h of exposure to calcitriol (100 nM). The increase was maximal following 6h (8 fold) and mRNA levels remained elevated for at least 24h. The increase in mRNA level was not due to increased mRNA stability as assessed by co-incubation with actinomycin D. Using specific inhibitors we showed that the effect of calcitriol is not mediated by ERK or Src that are known to be rapidly effected by calcitriol in keratinocytes.

Conclusions: The epidermal inflammatory response can be interpreted as notification of the presence of a threatening agent that results in recruitment of the host response to that threat. However, limitation of this response is necessary to avoid the deleterious consequences of chronic inflammation. Prostaglandins, produced by COX-2, play a dual role in the regulation of inflammation: augmentation at its early stages and attenuation during its final stage. The increase of the inducible form of COX by calcitriol may accentuate both actions.

Engineered Thyrotropin Variants with Increased In vivo Activity and High Serum Stability

Dr. Naiel Azzam¹ Dr. Fuad Fares²

¹*Department of Molecular Genetics, Carmel Medical Center and Faculty of Science, University of Haifa, Haifa, Israel*

²*Department of Molecular Genetics, Carmel Medical Center and Faculty of Science, University of Haifa, Haifa, Israel*

Introduction: Thyrotropin (TSH) is a heterodimeric hormone composed of a common α subunit shared with other glycoprotein hormones, and of a β subunit, which is unique and confers biological specificity. The action of this hormone leads to increased production of the thyroid hormones T3 and T4, which act on organs throughout the body to regulate growth and various metabolic processes.

The genes encoding the common α subunit and TSH β subunit were genetically fused in our lab, converting the hTSH dimer to a single-peptide chain (chimera). A C-terminal peptide of hCG β subunit (CTP) served as a linker between both genes, forming hTSH α -CTP- β single chain, which was proved to be biologically active in vitro systems. Two other chimeras were constructed:

1. hTSH α -CTP- β dimer: a dimeric molecule with CTP linker only on the β subunit.
2. TSH α - β single chain

Objective:

To evaluate the in vivo bioactivity and pharmacokinetic parameters of human thyrotropin variants.

Patients / Methods: The TSH variants were constructed using molecular biology technical methods. CHO cells were transfected with the vectors coding for the different TSH subunits for protein expression and stable clones were selected. The media, containing the secreted TSH constructs, were collected and concentrations of variants were measured. The biological activities of the thyrotropin variants were determined by measuring the levels of T4, the end product of thyrotropin action, in the circulation in mice, at selected time intervals. For this purpose a radioimmunoassay with TT4 RIA Human Neonatal T4 kit was used. For determination of serum stability of the engineered constructs, rabbits were injected with the variants. Blood samples were collected at certain time points after injection, and concentrations of variants in the serum were measured, using an immunoradiometric assay and a double antibody for thyrotropin.

Results: 8h after injection, the activity of hTSH WT dimer was undetectable. The activity of the hTSH α -CTP- β dimer showed a decrease of about 22% with regard to the measured initial amount, and reached about 78% of the activities of both single chain variants. The two later constructs preserved the same activities even after 8h. The primary results of serum stability indicated that the half-life of single chain constructs is about 70min while that of hTSH WT is about 30min.

Conclusions: 1. Single chain constructs are more active and more stable than dimeric constructs. 2. The CTP linker and/or the single chain construct, elongate the half-life of thyrotropin hormone.

Administration of leptin antagonist attenuates Concavalin A- and Pseudomonas Exotoxin A-induced experimental hepatitis and thioacetamide-induced hepatic fibrosis

Prof. Arieh Gertler¹ Ms. Gila Solomon¹ Mr. Muhammed Ali² Mr. Rafi Bruck²
Prof. Zamir Halpern² Dr. Eran Elinav²

¹*Institute of Biochemistry, Food Science and Nutrition, The Hebrew University of Jerusalem*

²*Gastroenterology and Liver Institute, Tel Aviv Sourasky Medical Center*

Introduction: It is well documented that leptin (LEP) augments inflammatory and profibrogenic responses in multiple murine models. Furthermore, leptin deficient ob/ob mice are protected from experimental hepatitis and develop severe liver steatosis but no fibrosis. Leptin's proinflammatory effect is most likely mediated by various T cell subsets which express leptin receptors (LEPRs) and are dependent for their function on leptin signaling, while its profibrogenic effect is mediated through activation of hepatic stellate cells (HSC). Those findings served as a rationale for use of our recently developed leptin antagonists as a putative therapeutic agent.

Patients / Methods: Two acute inflammatory experimental models, Concanavalin A (Con A) and Pseudomonas Exotoxin A (PEA) induced hepatitis, and chronic TAA induced fibrosis in mice were employed to test leptin antagonist's in vivo activity. In all experiments, mice were IP injected with daily doses of mouse leptin (0.5 – 1.0 mg/kg/day) and/or mouse leptin L39A/D40A/F41A mutein that acts as an antagonist (50 – 80 mg/kg/day). Serum alanine aminotransferase (ALT) was used as a marker of liver injury. In addition, metabolic markers such as cholesterol, TG as well as other inflammatory parameters were tested.

Results: Administration of Con A or PEA caused dramatic elevation in serum ALT and subsequent mortality. Both effects were augmented by simultaneous administration of leptin and significantly attenuated by co-administration of leptin antagonist. Administration of Con A alone caused up to four-fold increase in endogenous LEP and induced up to 30% increase in natural killer T (NKT) lymphocytes while administration of LEP antagonist reduced the level of those cells by 45%. Chronic administrations of TAA caused severe fibrosis and subsequent mortality. Co-administrations of LEP dramatically enhanced mortality, whereas administration of LEP antagonist totally abolished leptin's and partially attenuated TAA's effects. Prolonged administration of LEP antagonist did not result in any undesirable effect and its effect on body weight was marginal and statistically not significant.

Conclusions: Administration of leptin antagonists resulted in potent inhibition of leptin-mediated in vivo pro-inflammatory and profibrogenic effects and decreased dramatically the mortality of mice given Con A, PEA or TAA, thus indicating that leptin antagonists have a potential for clinical therapeutic usage. High excess of antagonist were required as both leptin and leptin mutein exhibit identical affinity toward LEPR. Therefore further development of leptin antagonists exhibiting higher affinity toward LEPR and prolonged in vivo persistence in circulation is being carried on.

Penicillamine induced dysmorphogenesis and hypothyroidism in healthy infants born to a mother with Wilson disease, and in Wilson disease patients

Prof. Aaron Hanukoglu¹ Dr. Batya Curiel¹ Dr. Drora Berkowitz³ Dr. Arieh Levine⁴ Dr. Mordehai Lorberboym⁵

¹*E. Wolfson Medical Center, Division of Pediatric Endocrinology, Holon and Tel-Aviv University Sackler School of Medicine*

³*Rambam Medical Center, Division of Pediatric Gastroenterology*

⁴*E. Wolfson Medical Center, Division of Pediatric Gastroenterology*

⁵*E. Wolfson Medical Center, Institute of Nuclear Medicine*

Introduction: Thyroid dysfunction has not been previously reported in Wilson disease patients. An infant born to a mother with Wilson disease, who was on penicillamine, had transient goitrous hypothyroidism, raising the question of whether penicillamine inhibits thyroid hormonogenesis in-utero. Penicillamine was previously shown to inhibit myeloperoxidase activity. Thyroid peroxidase that catalyzes the iodination and coupling of tyrosine, shares amino acid sequence homology with myeloperoxidase. We hypothesize that penicillamine may inhibit thyroperoxidase activity both in-utero and in Wilson disease patients.

Patients / Methods: Two siblings born to a mother with Wilson disease who received penicillamine during pregnancy, and 5 patients (the mother of two siblings, two brothers and two unrelated children aged 10-13 y) who were on penicillamine for 3-5 years. Penicillamine was discontinued in four patients and put on zinc therapy. Thyroid function tests (on penicillamine and zinc therapy respectively), thyroid antibodies and thyroid radionuclide imaging (Tc- 99m) were performed in all subjects. Thyroid uptake and thyroid receptor antibodies were performed in some subjects.

Results: Two male siblings born to a mother with Wilson disease had goitrous hypothyroidism diagnosed at 15 and 36 days of age (FT4=undetectable, TSH > 100 mU/L) with negative thyroid stimulating and blocking antibodies. Sib. 1: On eltroxin, the thyroid function tests normalized within few weeks and the goiter disappeared. Eltroxin was discontinued at 4 y. of age. Sib. 2: radionuclide imaging (enlarged thyroid gland with increased uptake) was consistent with goitrous hypothyroidism. L-T4 therapy stopped at 2.5 mo. of age. Four additional WD patients had subclinical hypothyroidism on penicillamine (mean± SD, TSH= 4.3±03, normal=0.25-4 mU/L). On zinc therapy, TSH decreased significantly in all (TSH =2.2± 0.29 mU/L, P< 0.0001). FT4 levels were normal.

Conclusions: Penicillamine inhibits thyroperoxidase activity (iodination and coupling) in-utero and in Wilson disease patients, resulting in transient goitrous hypothyroidism in healthy infants and sub-clinical hypothyroidism in Wilson disease patients. In pregnant mothers with Wilson disease, zinc therapy may prevent goitrous hypothyroidism in newborns.

Medullary thyroid carcinoma in Israel

Dr. Anat Jaffe¹ Dr. Micha Barchana^{2,4} Dr. Liana Tripto Shkolnik¹ Dr. yoel toledano¹ Dr. Michael Hopp³

¹Endocrinology & Diabetes Unit, Hillel Yaffe Medical Center, Hadera

²Israel National Cancer Registry, Ministry of health, Jerusalem, Israel

³Tel Aviv University; & HOP research

⁴School of public Health, Haifa University

Introduction: Background: A marked increase in thyroid cancer (TC) incidence over the last two decades, mainly due to papillary carcinoma (PTC) has been noted in several different Israeli subpopulations. The reasons for this rise in TC incidence are probably multi-factorial and may relate partly to an increased diagnostic vigilance [A Lubina, thyroid 2006]. Our hypothesis is that changes in clinical practice regarding thyroid nodules that affected incidence of PTC, might affect as well Medullary Thyroid Cancer (MTC) identified incidence, patient's age of diagnosis, stage of disease and survival rates.

Patients / Methods: Records of 281 MTC patients (diagnosed between 1982 and 2005), were retrieved from the Israel National Cancer Registry. The record includes data on age, place of birth, ethnicity, date of diagnosis and place of operation for most cases. Data on the type and magnitude of the operation were added to the registry only in 1992. Details on tumor pathology concerning tumor size and number of lymph node tested and involved were recorded from 1997. All data were coded and recoded into quantitative variables, and were then analyzed by Spsswin and spsspc.

Results: The cohort includes; 60.1% females, and 39.9% males. Mean age at diagnosis for both genders is 51.8 ± 18.9 years with no gender effect, 89.7% are Jewish and 7.1% are Arab. At the time of this report 69% of patients are alive and the average survival is 4.1 ± 4.4 years. Tumor size is significantly smaller in females 2.2 ± 1.3 cm vs. 3.3 ± 2.2 in males. Accurate information regarding stage at diagnosis is available for 60.1% records. Of these: 11.7% patients, 8.9%, 33.5% and 6% were diagnosed at stage 1 to 4 respectively. Forty six percent of the records contained information about the scope of the operation. 19.4% patients only had total thyroidectomies (tTY); 25.6% patients had tTY with limited lymph node dissection (LNd), 22% patients had tTY with radical/modified LNd. The other 32.6% reported patients had lobectomy \pm LNd. The extent of LNd was dependent on the local medical center and was not correlated with the final staging of the disease, with the Soroka MC being the most radical. Moreover, the magnitude of the operation was not changed along the reported years. These findings have several limitations as there is no active follow-up on treatments and some of the patients probably had a second operation after final diagnosis was made.

Age of diagnosis was tested against gender, region of medical center and place of birth.

Gender and region were not significant while place of birth was highly significant, for Israel-born group being the youngest. The age-standardized incidence rate (ASR per 105 persons) of MTC is low <0.5 and not significantly different over the observed period.

Conclusions: New trends in thyroid nodule management had no impact on the diagnosis and treatment of the rare MTC malignancy of the thyroid.

Complications of antithyroid agents: A summary: 1968-2007

Prof. Menachem Shapiro¹ Dr. Rosani Ness Abramov¹ Dr. Gavriel Solomen¹ Dr. Mark Nevin² Dr. Lima Witz (z"l)¹ Prof. Louis Shenkman¹ Dr. Dan Nabriski¹

¹*Meir Hospital, Kfar Sava*

²*Laniado Hospital Natanya*

Introduction: The complications of antithyroid agents are known. We have summarized our experience with some more memorable complications occurring with these agents

Patients / Methods: We have treated several hundreds of patients referred to endocrine clinics with these agents over the last 39 years. The patients presented in this communication represent those with a special aspect.

Results: Two patients developed agranulocytosis (A) and died during hospitalization. Two patients had 2 febrile episodes within a week. A was detected on the second episode. A developed in a single patient during a relapse of his disease treated with the same agent. A developed in a single subject following administration of both PTU and Mercaptozle. A developed after two years treatment with PTU in one subject. One patient developed A and jaundice. Drug fever developed in one patient. A single patient became jaundiced with a maximal bilirubin of 20mg. He responded to I 131 while still jaundiced.

Over 20 patients developed polyarthralgia, In one, RA develop a year after cessation of the agent. A drug eruption developed in one subject but PTU was continued. The rash resolved but this was followed by the appearance of polyarthralgia. A single patient developed a pleural effusion with detection of LE cells in the fluid. On stopping PTU the effusion resolved.

A single patient developed hypoglycemia with administration of PTU. This resolved on cessation of tPTU

Conclusions: The endocrinologist will meet many of the complications of these agents. He or she One must be sure the patients comprehends the potential severity of these reactions. One cannot rely on the family physician to relay the message. Most of the reactions are minor but some may be life threatening and need to be remembered.

Loss of thyrotropin set points in Down syndrome subjects as a possible mechanism for their hypothyroidism

Dr. Joseph Meverovitch^{1,2} Dr. Michael Sherf^{1,3} Mrs. Felice Antebi¹ Prof. Zeev Hochberg⁴

¹Health Planning and Policy Wing, Clalit Health Services, Tel Aviv

²Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Affiliated to Tel Aviv University Sackler School of Medicine, Tel Aviv

³Department of Family Medicine, Ben Gurion University, Beer-Sheva

⁴Division of Endocrinology Meyer Children's Hospital and Technion, Haifa

Introduction: Background: The prevalence of thyroid disease is high in Down's syndrome (DS) subjects.

Objectives: To understand the thyroid disease in DS.

Hypotheses: 1. A subset of DS subject have a thyroid disease 2. Thyroid disease is an integral part of the syndrome.

Patients / Methods: We used a centralized computerized database of 3.7 million subjects insured by Clalit Health Medical Organization (CHMO) to analyze thyroid functions tests (TFT) of DS as compared to the general population (GP). DS was identified using database records, based on primary physician records. Of the GP patients those with a diagnosed thyroid disease or those receiving treatment with L-thyroxine, antithyroid drugs, lithium, amiodarone or interferon were excluded.

Results: DS was diagnosed in 1,424 patients (0.04 %), 54.85% males. 358 (25.4%) were diagnosed as having a "thyroid disease", 32 (2.25%) hyperthyroidism and 326 (22.9%) hypothyroidism. The distribution of DS' TSH levels prior to treatment shows a clear shift of the curve to higher levels, with a distribution mode of 1.6mU/L (2.38 ± 3.99 , mean \pm SD) in GP and 3.6mU/L in DS (4.18 ± 3.2), ($p < 0.0001$), (GP random sample $n=46,149$). The FT4 distribution mode is 13.8 (14.86 ± 2.84) in GP and 15.0 in DS (15.24 ± 8.26 , NS). The regression line of FT4 against TSH was negative in GP ($TSH=13.97-0.67FT4$, $F < 0.0001$) but surprisingly positive in DS ($TSH=1.84+0.20FT4$, $F < 0.0001$).

Conclusions: The distribution shift explains most cases of diagnosed hypothyroidism in DS. Hypothyroidism is an integral part of DS and not a phenomenon of a subset of subjects. DS patients lose the thyrotrope set point for feedback by thyroid hormones, and therapy should be contemplated in all.

Neonatal hyperthyrotropinemia (HT): population characteristics, diagnosis, management and outcome after cessation of therapy

Dr. Amnon Zung¹ Dr. Shiri Barkan¹ Dr. Yardena Tenenbaum-Rakover² Prof. Aaron Hanukoglu³ Prof. Eli Hershkovitz⁴ Prof. Tzvy Bistrizer⁵ Dr. Orit Pinhas-Hamiel⁶ Prof. Zvi Zadik¹

¹*Pediatric Endocrinology Unit, Kaplan Medical Center, Rehovot*

²*Pediatric Endocrinology Unit, Ha'Emek Medical Center, Afula*

³*Division of Pediatric Endocrinology, E. Wolfson Medical Center, Holon*

⁴*Pediatric Endocrinology Unit, Soroka Medical University, Beer-Sheva*

⁵*Pediatric Endocrinology Unit, Assaf-Harofe Medical Center, Tzrifin*

⁶*Pediatric Endocrinology Unit, Safra Children's Hospital, Chiam Sheba Medical Center, Tel-hashomer*

Introduction: HT is defined by normal plasma levels of T4 in association with high plasma TSH. Neonatal screening for congenital hypothyroidism (CH) in Israel measures blood concentration of T4, therefore, neonates with HT are usually not detected. Although the clinical significance of HT is not fully understood, many clinicians treat these patients for the first few years of life. On cessation of therapy, some of the patients remain euthyroid (transient HT; t-HT), while others show the pattern of persistent HT (p-HT).

We aimed to evaluate the diagnosis pattern, patients' characteristics and principles of treatment in neonates with both types of HT, compared with CH neonates.

Patients / Methods: We conducted a retrospective, multi-center study in six pediatric endocrine units. All neonates who were diagnosed with either HT or CH from 1995 to 2006 were included. HT was diagnosed based on confirmatory sample that followed abnormal screening or initial sample taken on clinical ground. Infants to mothers with thyroid disease were excluded.

Results: The study included 25 patients with HT and 53 patients with CH. In 14 HT neonates (56%) diagnosis was made based on clinical signs compatible with hypothyroidism, compared with 15 neonates (28%) with CH ($p=0.032$). Eleven cases of HT were diagnosed based on abnormal thyroid screening results. 36% of HT patients were born premature compared with 13% CH patients ($p=0.04$). HT neonates were diagnosed and started therapy later than CH neonates. On thyroid imaging, 71% of HT patients had normal results compared with 30% CH patients ($p=0.01$).

HT patients had a better control during treatment: 56% of the measurements in HT patients were normal (both FT4 and TSH) compared with 24% in CH patients ($p<0.001$). In addition, elthorxin dosage (when both TSH and FT4 were normal) was lower in HT patients after 1.5 years, compared with CH patients.

On cessation of therapy, 11 HT patients (44%) became euthyroid. Thyroid imaging was the only parameter that distinguished between HT sub-groups: all 9 patients with t-HT who studied had normal thyroid imaging compared with 3 out of 8 patients with p-HT ($p=0.023$). FT4 levels before and during treatment were similar between HT groups, but they were significantly higher in t-HT than in p-HT after cessation of therapy.

Conclusions: Prematurity, relatively low requirements of elthorxin and normal thyroid imaging are more prevalent in HT than in CH. Transient form of HT is more common than previously reported in neonates with HT. We suggest that t-HT demonstrates a maturation defect in thyroid gland (sometimes associated with prematurity), whereas p-HT patients maintain a different set-point of sensitivity to T4.

Hyponatremia in the elderly is associated with hypercortisolemia rather than adrenal insufficiency

Dr. Tania Winchester¹ Prof. Moshe Sonnenblick^{1,2} Dr. Gabriel Munter²

¹Shaare Zedek Medical Center, Geriatrics Department

²Shaare Zedek Medical Center, Internal Medicine Department

³Ben Gurion University

Introduction: Hyponatremia (HN) is a common cause of hospitalization in the elderly. Inappropriate secretion of Antidiuretic Hormone is the most frequent etiology of HN. HN could also be caused by Hypocortisolism (AI), mimicking SIADH. In a previous retrospective study, AI was found in 15% of HN patients. The aim of this study was to determine the frequency and characteristics of AI in elderly patients with HN.

Patients / Methods: This prospective study was performed during five consecutive months in the medical ward of Shaare Zedek Medical Center. Patients over 65 years old with Na⁺ 135 meq/L hospitalized during the same period were selected as controls. Patients suffering from overwhelming infection and neoplastic disease were excluded. Plasma cortisol levels were determined before, 30 and 60 minutes after i.v. administration of 1 mcg ACTH. Basal cortisol levels were performed during the morning. Subjects with cortisol levels over 20mcg/dl (550 nmol/L) at any time during the test were considered to have normal cortisol secretion.

Results: 30 patients were recruited in each group. HN: age 83 ± 7 and Na 125 ± 5 meq/L. Control: age 79.3 ± 8 and Na 139.8 ± 2 meq/L. Stimulated cortisol levels were compatible with normal adrenal function in all subjects from both groups. Baseline cortisol was sufficient to exclude AI in 2/3 of HN patients. Cortisol secretion was higher in HN vs controls, both in the basal state (21.2 ± 7.8 and 13.8 ± 4.9 mcg/dl respectively. $p < 0.0001$) and after stimulation (33.8 ± 9.2 and 28.3 ± 5.8 mcg/dl respectively. $p < 0.05$). The increment in cortisol levels after stimulation was similar in the 2 groups (13.1 ± 7.1 vs 14.6 ± 5.6 mcg/dl), with peak cortisol occurring at 30 min. after ACTH.

Conclusions: 1) AI is not a common cause for HN in the elderly. In most HN patients AI can be excluded by baseline cortisol. 2) HN is associated with relative hypercortisolemia. This suggests that HN may be associated with greater stress and/or higher levels of ADH, a CRH secretagogue, compared with normonatremic subjects.

5 α -Reduced Steroid Metabolites Interfere With Steroidogenesis In The Human Adrenal Tumor Cells H295R

Dr. Michal Lahav¹ Ms. I. Karina Tereshenkov¹ Prof. Zeev Blumenfeld² Dr. Carlos Knopf³

¹Unit of Endocrinology, The Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa

²Ob/Gyn Dept., The Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa

³Laboratory of Metabolic Diseases and Steroid Metabolome, Department of Clinical Biochemistry, Rambam Medical Center, Haifa

Introduction: Hyperandrogenism in women originates in the adrenal or ovary, but secondary influences of one gland on the other make diagnosis difficult. We used cultures of H295R, human adrenal tumor cells that synthesize all the adrenal steroid hormones, to look for direct interference of androgens with adrenal steroidogenesis.

Patients / Methods: Cells were grown with Ultrosor, but experiments were conducted in medium in which BSA replaced Ultrosor. After 24 h incubation with exogenous steroids, the medium was collected, and the incubation continued for 3 h in medium devoid of added steroids. Steroids were analyzed by RIA or gas chromatography-mass spectrometry (GC-MS).

Results: We have previously reported that several androgens at concentrations found in plasma had no effect on cortisol production. The mRNAs of 17- and 21-hydroxylases were also unaffected. However, since abnormal accumulation of endogenous intra-adrenal steroid metabolites, secondary to hyperandrogenism, could occur, we examined androgens at up to 5 μ M, a concentration relevant intra-adrenally. RIA was used to measure progesterone (P), 17 α -hydroxy-P (17-OH-P) and cortisol (C) in the medium collected 3 h after androgen removal. Only 5 α -dihydrotestosterone (DHT) and 5 α -androstanedione (Andione) were inhibitory (at μ M and hundreds of nM, respectively). DHT inhibited mostly between P and 17-OH-P, whereas Andione inhibited mostly between 17-OH-P and C. 5 α -Dihydro-P slightly reduced the 17-OH-P/P ratio. Using GC-MS, we estimated the production of 12 steroids, and are in the process of measuring 15 more, after 24 h incubation. In the control, the most abundant steroids were C, P and androstenedione. When DHT and Andione at 5 μ M were added, we found that the exogenous steroids were partly metabolized. The ratios among the measured steroids suggested that both androgens inhibited endogenous androgen production. Andione greatly increased the accumulation of 17-OH-P, with a minor decrease in the C/11-deoxy-C ratio. The ratio 5 α -dihydro-P/P, indicative of 5 α -reductase activity, was two orders of magnitude greater with both androgens, compared to the control. DHT and testosterone at 50 nM had little effect.

Conclusions: In PCOS, 5 α -reductase activity was shown to increase, and follicular fluid was found to contain elevated Andione, which inhibited aromatase. In our study, steroidogenesis was inhibited by similar Andione concentrations, and production of 5 α -dihydro-P greatly increased. Our working hypothesis is that in H295R cells androgens may increase the activity of 5 α -reductase, resulting in high concentrations of intra-adrenal 5 α -reduced inhibitory metabolites.

Bone Turnover after Alendronate Dose Reduction Following Prolonged Standard Full Dose Treatment in Postmenopausal Osteoporosis Patients

Dr. Elena Segal¹ Mrs. Zila Shen-Or² Mrs. Batia Raz² Prof. Sophia Ish-Shalom^{1,3}

¹*Metabolic Bone Diseases Unit, Rambam Medical Care Campus, Haifa*

²*Endocrine Laboratory, Rambam Medical Care Campus, Haifa*

³*The Bruce Rappaport Faculty of Medicine, Technion -Israel Institute of Technology, Haifa*

Introduction: Effect of bisphosphonate treatment on fracture risk reduction is mainly due to decrease in bone turnover. There are growing, though yet unsubstantiated, concerns about possible risks of life long bisphosphonates administration. On the other hand discontinuation of alendronate for 12 months led to increase of bone turnover. The effect of treatment with decreased bisphosphonates dose on bone turnover is unknown. The aim of this study was to evaluate bone turnover in patients treated with monthly or biweekly dose of 70 mg of alendronate and compare it to the standard 70 mg/wk dose.

Patients / Methods: Postmenopausal patients with stable BMD and absence of fractures during standard weekly treatment (ST) for 4-5 years were switched to biweekly or monthly dose, following personal preference of each patient. Bone turnover (BT) was assessed by serum total procollagen type I amino-terminal peptide (P1NP) and serum collagen beta cross-laps (CTX) in patients on ST regimen and at 12 and 24 months of biweekly (B) or monthly (M) therapy

Results: 132 postmenopausal women were enrolled in the study: 65 on ST, 7 on B for one year, 21 on M for one year, 16 on monthly for two year (MTY). Fourteen patients were tested while untreated (NT), 9- were on calcium and vitamin D supplementation (CD).

P1NP levels were 24 ± 14.1 ; 27.7 ± 11.3 ; 24 ± 8.9 ; 26 ± 10 in ST, B, M and MTY, respectively.

CTX - 0.2 ± 0.11 ; 0.28 ± 0.19 ; 0.21 ± 0.1 ; 0.19 ± 0.11 in ST, B, M and MTY, respectively. P1NP in CD, NT was 43.14 ± 23 ; 47.2 ± 20 ; CTX- 0.36 ± 0.19 ; 0.39 ± 0.23 , respectively.

Premenopausal values of bone turnover markers are: P1NP 15.13-58.59ng/ml; CTX 0.299 ± 0.14 ng/ml.

There was no significant difference in BT between NT and CD patients. P1NP didn't differ between treatment groups, as well as between treated and NT patients. CTX levels were significantly lower in all treatment groups, compared to CD and NT, $p=0.005$ for ST, 0.026 for B, M, MTY

Conclusions: Biweekly and monthly regimens of alendronate treatment after a prolonged standard weekly treatment keeps bone turnover stable in the premenopausal range. It could be a cost effective and possibly safe option for prolonged treatment of postmenopausal osteoporosis patients.

Parathyroid Hormone Selective Venous Sampling (SVS): Surgical Adjunct to allow Minimally Invasive Parathyroidectomy in Sestamibi Scan Negative Patients

Prof. Michael Krausz^{1,4} Prof. Sophia Ish-Shalom^{2,4} Dr. Elena Segal² Dr. Amos Ofer³ Dr. Ahuva Engel³ Dr. Michal Mekel¹

¹*Department of Surgery A', Rambam Medical Care Campus, Haifa*

²*Metabolic Bone Disease Unit, Rambam Medical Care Campus, Haifa*

³*Invasive Radiology Unit, Rambam Medical Care Campus, Haifa*

⁴*Technion-Israel Institute of Technology, Haifa*

Introduction: Minimal invasive parathyroidectomy (MIP) has become the procedure of choice for treatment of primary hyperparathyroidism (PHPT) induced by single enlarged parathyroid adenoma. A prerequisite for this procedure is preoperative localization of a single enlarged gland by the Sestamibi scan and/or ultrasound performed by an experienced radiologist. In Sestamibi scan and ultrasound negative patients with PHPT conventional bilateral neck exploration is usually chosen.

Patients / Methods: Between April 1999 and January 2007, 306 patients with biochemically verified hyperparathyroidism (HPT) were surgically treated at the Rambam Medical Center. Our series includes 254 patients with PHPT and 52 with secondary HPT. MIP was performed in 188 of the 209 (89.9%) of patients with an adenoma. In 75 (35.8%) patients this procedure was performed under local or regional anesthesia. From January 2005, all patients with PHPT and a negative Sestamibi scan negative or ultrasound negative or non conclusive patients underwent PTH selective venous sampling (SVS) as part of their initial preoperative workup.

Results: A total of 248 of the 254 patients with PHPT (97.6%) were cured of the disease. SVS was performed in 30 patients. In 10 patients SVS was performed for recurrent or persistent PHPT. Out of 20 patients with a negative Sestamibi scan, preoperative SVS correctly located a single parathyroid adenoma in 15 patients (75%), and allowed successful MIP in 11 (73.3%) of the 15 patients.

Conclusions: Preoperative PTH selective venous sampling can be considered in Sestamibi scan negative patients with PHPT. When a significant PTH gradient is demonstrated in SVS, we recommend MIP as an alternative to conventional wide bilateral cervical exploration.

Plant-derived micronutrients inhibit estrogen signaling in mammary cancer but not in bone osteoblast-like cells

Mrs. Anna Veprik¹ Dr. Marina Khanin¹ Dr. Keren Hirsch¹ Dr. Michael Danilenko¹ Prof. Yoav Sharoni¹ Prof. Joseph Levy

¹*Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University and Soroka Medical Center of Kupat Holim, Beer-Sheva*

Introduction: The opposing processes, bone formation and bone resorption, are regulated by several hormones including 1,25(OH)₂D₃ and estradiol (bone formation) and glucocorticoids (bone resorption). We have previously found that carotenoids, polyphenols and isothiocyanates support bone formation by increasing the level of bone-forming proteins such as alkaline phosphatase (ALP) and osteoprotegerin (OPG). Moreover, these micronutrients augmented the positive effect of 1,25(OH)₂D₃ on the level of the bone markers while preventing the glucocorticoid-induced decrease in OPG level. In relation to the effect of the tested dietary micronutrients on estrogen activity, we previously found that in mammary and endometrial cancer cells, they inhibited estrogen signaling. Thus, it was important to determine whether these micronutrients specifically inhibit the harmful signals of estrogen in cancer cells and not its positive action in bone cells.

Patients / Methods: .

Results: MC3T3 mouse calvaria osteoblast-like cells were transfected with a luciferase reporter construct containing estrogen-response element. We found that various carotenoids (or their active derivatives), polyphenols (carnosic acid and curcumin) and the isothiocyanate sulforaphane have no effect or even stimulated the estrogen-induced reporter gene activity. In contrast, these compounds inhibited, as expected, transactivation of the same reporter gene when transfected to T47D mammary cancer cells. To understand the differential effect on estrogen signaling in the two cell types we examined the role of the Nrf2 transcription factor in modulating estrogen activity in the osteoblast cells. We previously found that this transcription factor is activated by the dietary compounds in both mammary cancer and bone cells and is involved in the inhibition of estrogen signaling in the cancer cells. To test the role of Nrf2 in the bone cells, we positively modulated its activity by the classical activator tert-butyl hydroquinone (tBHQ), which resulted in stimulation of the estrogen-induced reporter gene activity similar to that achieved with carotenoids, polyphenols or isothiocyanates. In contrast, it inhibited, as expected, the reporter activity in the mammary cancer cells.

Conclusions: Dietary micronutrients, which inhibit estrogenic activity in cancer cells, do not inhibit but even stimulate estrogen signaling in bone cells. Although this appears to be a SERM-like effect, the mechanism probably does not result from direct interaction of the compounds with the receptors but rather related to activation of other transcription factors.

Immune cytokines modulate the levels of vitamin D receptor in keratinocytes

Ms. Esther Ziv^{1,2} Prof. Ruth, Ruth Koren^{1,2} Dr. Amiram Ravid^{1,3}

¹*Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel-Aviv University*

²*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University*

³*Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University*

Introduction: The epidermis contains an autonomous vitamin D endocrine system, including the enzymes needed to produce and degrade the hormonally active metabolite of vitamin D, calcitriol and the nuclear vitamin D receptor (VDR). Calcitriol binds to the VDR that forms a hetero-dimer with RXR to modulate the expression of vitamin D responsive genes. In view of the well-established anti-inflammatory role of vitamin D in the skin, we aimed to examine the notion that the efficacy of the epidermal vitamin D endocrine system is altered during epidermal inflammation.

Patients / Methods: Our experimental model were immortalized HaCaT cells cultured in the absence of exogenous growth factors or active mediators that represent the mitotic population of basal keratinocytes. The inflammatory state was simulated by exposure to TNF α (10 ng/ml for 24 hours) or to interferon-g. Levels of VDR and RXR were assessed by immunoblotting. mRNA levels were assayed by real-time PCR based on TaqMan technology.

Results: Exposure of HaCaT cells to TNF significantly and consistently increased VDR levels (62 \pm 10% \pm , $p < 0.0001$) in a dose dependent manner without affecting RXR levels, while exposure to interferon-g decreased VDR levels. Inhibition of PKC using the pan-PKC inhibitor, bisindolylmaleimide, increased VDR levels, which were not further increased by TNF treatment. Inhibition of the ERK pathway with U0126 increased VDR levels in control cells, while attenuating its upregulation by TNF. Inhibition of p38-MAPK did not affect VDR levels. The upregulation of VDR by TNF is at least partially mediated by c-Jun N-terminal kinase as inhibition this kinase decreased VDR levels in control cells and blocked its upregulation by TNF. The upregulation of VDR by TNF was manifested also at the mRNA levels, detectable already after 8 hours and maximal between 16 and 24 hours of TNF treatment. The presumed increased efficacy of calcitriol signaling due to upregulation of the VDR was ascertained by demonstrating increased mRNA levels of the VDR target gene, 25-hydroxyvitamin D 24-hydroxylase, following treatment with sub-maximal concentrations of calcitriol.

Conclusions: The demonstration that exposure to the inflammatory cytokines TNF and interferon-g alters the sensitivity of keratinocytes to calcitriol are in line with the notion that the epidermal vitamin D endocrine system takes part in the regulation of epidermal inflammation.

GnRH Agonist Treatment In Girls With Precocious Puberty Does Not Compromise Post-Pubertal Uterine Size

Dr. Liat de Vries^{1,4} Dr. Hadassa Goldberg-Stern^{2,4} Prof. Moshe Phillip^{1,4} Dr. Avi Ben-Haroush^{3,4}

¹*Institute for Endocrinology and Diabetes, Schneider's Children Medical Center in Israel*

²*Epilepsy Center, Schneider's Children Medical Center in Israel,*

³*Helen Schneider Hospital for Women, Rabin Medical Center, Beilinson Campus*

⁴*Sackler school of Medicine, Tel-Aviv University*

Introduction: Prompted by findings of a hypoestrogenic state in girls during prolonged treatment with GnRH agonist and a close association of estradiol serum concentrations with uterine volume in puberty, this study sought to evaluate uterine and ovarian size in girls with precocious puberty (PP) during and after treatment and the effect of age or duration of treatment.

Patients / Methods: Patients with idiopathic central PP before (n=75), during (n=41), or after (n=30) treatment with GnRH agonist underwent transabdominal pelvic ultrasound examination. Findings were compared to those in 69 girls with epilepsy before initiation of anticonvulsant treatment.

Results: The girls with PP had significantly greater uterine and ovarian volumes before, during and after treatment with GnRH agonist than the controls, after adjusting for age at examination, weight, height, and pubic and breast status. The average interval between the last treatment and the ultrasound examination was 1.3 years. There was no significant correlation between age at first treatment and uterine volume after treatment. Uterine volume decreased during treatment. There was a significant negative correlation between treatment duration and uterine volume after treatment. Nevertheless, mean uterine volume was still greater in the treated group than in the control group.

Conclusions: The iatrogenic hypoestrogenic state in treated girls with PP does not compromise post-pubertal uterine size.

Abstract Code: P60

Nutrition-induced catch-up growth increases hypoxia inducible factor 1a mRNA levels

Ms. Naomi Even Zohar¹ Mrs. Jasmine Jacob² Dr. Ninnette Amariglio² Prof. Gideon Rechavi² Mrs. Olga Potievsky¹ Prof. Moshe Phillip¹ Dr. Galia Gat-Yablonski¹

¹*Institute for Endocrinology and Diabetes, National Center for Juvenile Diabetes, Schneider Children's Medical Center of Israel, and Felsenstein Medical Research Center, Petach Tikva*

²*Laboratory of Molecular Biology, Institute of Hematology and Cancer Research Center, Sheba Medical Center, Tel Hashomer*

Introduction: Catch-up growth is a well-known phenomenon; however, the local pathways at the epiphyseal growth plate that govern this process remain largely undescribed

Patients / Methods: Prepubertal Sprague-Dawley rats were subjected to 10 days of 40% food restriction, followed by a renewal of the regular food supply to induce catch-up growth. The animals were weighed daily, and their humeral length was measured at sacrifice. The proximal tibial epiphyseal growth plates (EGPs) were taken, and findings were compared with EGPs from animals fed ad libitum and animals under food restriction. Gene expression profile in the growth plates was examined using microarrays, and the expression levels of selected genes were validated by real-time PCR. To localize gene expression in different growth plate zones, microdissection was used. Protein levels and localization were examined using immunohistochemistry.

Results: The expression level of 550 genes decreased during food restriction and increased during catch-up growth, starting already one day after refeeding. Most of the enriched functional categories within this group were involved in synthetic functions, namely macromolecule metabolism, RNA processing and translation, protein transport, secretion and degradation. Among these genes we found HIF-1a mRNA, as well as several of its downstream targets. Immunohistochemistry showed a similar pattern for HIF-1a protein levels. Additionally, HIF-1a mRNA and protein levels were higher in the proliferating than the hypertrophic zone, and this distribution was unaffected by nutritional status.

Conclusions: Nutrition appears to have a profound effect on gene expression level during growth plate growth. The findings suggest an important role for HIF-1a mRNA in the prepubertal growth plate and its response to nutritional manipulation

Growth pattern and final height after cessation of gonadotropin-suppressive therapy in girls with central sexual precocity

Dr. Liora Lazar¹ Dr. Anna Padoa² Prof. Moshe Phillip¹

¹*Institute for Endocrinology and Diabetes, Schneider Children's Medical Center of Israel, Petah Tiqva 49202, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel*

²*Department of Obstetrics and Gynecology, Assaf Harofe Medical Center, Zerifin, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978 Israel*

Introduction: Objective: The impact of growth occurring after discontinuation of gonadotropin-suppressive therapy (GnRHa) on final height (FHt) of girls with sexual precocity is well established. Our aim was to find out if post treatment height gain differs in girls diagnosed at various ages, and to assess its influence on FHt outcome

Patients / Methods: Design: Clinical and anthropometric features of 115 girls with sexual precocity (22 - diagnosed before chronological age (CA) of 6 years, 38 - between ages 6-8, and 55 - between ages 8-9) treated with GnRHa from Tanner stage 2-3 to CA 11-12 and bone age (BA) 12-12.5 were studied. Data on post-treatment course and FHt of the 3 groups were compared.

Results: Despite comparable BA at cessation of treatment (12.1 ± 0.5 , 12.4 ± 0.5 and 12.4 ± 0.4 yrs), similar time to resumption of puberty (0.6 ± 0.7 , 0.5 ± 0.7 and 0.5 ± 0.7 yrs), and age at menarche (12.6 ± 0.5 , 12.6 ± 0.6 and 12.7 ± 0.9 yrs), height gain from cessation of therapy to FHt (10.8 ± 2.5 vs. 7.2 ± 4.3 and 5.5 ± 2.3 cm) was greater, and time to epiphyseal fusion (2.4 ± 0.4 vs. 2.0 ± 0.5 and 1.5 ± 0.4 yrs) was longer in the younger CPP compared to older CPP ($p < 0.05$) and EFP ($p < 0.001$) groups. The percentage of residual growth predicted at discontinuation of treatment was achieved only by the younger CPP group ($6.7 \pm 1.6\%$ vs. $6.6 \pm 1.6\%$) while in older CPP and in EFP groups, it was significantly lower ($4.6 \pm 2.7\%$ vs. 6.2 ± 1.6 and $3.6 \pm 1.5\%$ vs. $6.3 \pm 1.5\%$, respectively). FHt of these two groups was compromised compared to FHt predicted at discontinuation of treatment (157.9 ± 5.1 vs. 161.4 ± 6.5 ; 153.9 ± 4.6 vs. 158.4 ± 5.8 cm).

Conclusions: Girls with sexual precocity diagnosed after the age of 6 exhibit earlier epiphyseal fusion with diminished post-treatment height gain and compromised FHt. Since recovery of gonadal axis was similar in all girls, the differences were probably due to intrinsic changes in the growth plate occurring before introduction of treatment. Prediction of residual growth at discontinuation of treatment is unreliable in these girls.

Glucagon stimulation test for childhood GH deficiency – timing of the peak is important

Dr. David Strich¹ Dr. Nahum Terespolsky¹ Dr. David Gillis²

¹*Pediatric Specialty Clinic, Endocrinology and Diabetes, CHS*

²*Department of Pediatrics, Pediatric Endocrinology Clinic, Hadassah University Hospital, Ein-Kerem, Jerusalem*

Introduction: The glucagon stimulation test (GST) is used for the evaluation of GH deficiency. A peak GH less than 7-10 ng/ml is considered evidence of deficiency. Proof of deficiency requires a confirmatory test with a different secretagogue since some GH sufficient patients do not respond to glucagon. The classic GST utilizes 7 samples taken before administration of glucagon and then every 30 minutes for 3 hours. The only known significant result is the maximum value of the 7 samples (peak).

Hypothesis: Unusual timing of the peak might indicate abnormal GH secretion.

Patients / Methods: We retrospectively evaluated 222 GST's from a single pediatric endocrine testing center in Jerusalem. If GH levels were not above 10 ng/ml at any of the 7 standard sampling times the test was considered evident of GH deficiency (=positive). If the peak occurred at 90' or 120' regardless of positivity or negativity the test was termed "central". If it occurred at any other time (i.e. at 0', 30', 60', 150' or 180') it was termed "peripheral". We tested whether patients with peripheral GST's had a higher chance to be GH deficient based on a confirmatory test. We compared the growth velocity standard deviation scores (GVSDS) of patients with "central" and "peripheral" GST's who had a normal confirmatory test.

Results: 167/222 GSTs (75.2%) were central. Among peripheral tests 32/222 (14.4%) peaked at 0', 30 or 60', 25/222 (11.2%) at 150' and only 3/222 (1.3%) at 180'. 55/222 GSTs (24.8%) were positive. 21/55 (38.1%) peripheral, 34/55 (61.8%) central. 30/55 (55%) patients with positive GSTs were diagnosed as GH deficient by a confirmatory positive clonidine or arginine stimulation test. 16/21 (76.2%) patients with a positive "peripheral" GST had a positive confirmatory test compared with 14/34 (41.1%) patients with a positive "central" GST, ($p < 0.025$). Data regarding GVSDS were available for 24/25 GH sufficient patients with positive GSTs (18 central and 6 peripheral) but negative confirmatory tests. The average GVSDS for central GSTs was 0.1 (range -1.54-3.95), compared with -1.58 (range: -3.1 - 1.76) for peripheral tests, $p = 0.03$.

Conclusions: A) Patients with "peripheral" GSTs appear to be a distinct group. They grow less well and have a significantly higher chance to be diagnosed as growth hormone deficient. These patients might have defective GH secretion despite a negative confirmatory test.

B) The standard GST can be shortened to 150 minutes with 6 samples, thus reducing the risk of late hypoglycemia

Relationship between antidepressants and the IGF-1 system in the brain: Possible role in cognition

Dr. Michal Taler¹ Mrs. Nurit Grunbaum-Novak¹ Prof. Hagit Cohen² Prof. Abraham Weizman¹ Dr. Irit Gil-Ad¹ Prof. Ronit Weizman³

¹*Laboratory of Biological Psychiatry, Felsenstein Medical Research Center Institute, Campus Rabin, Petah-Tiqva and Tel-Aviv University*

²*Mental Health Center, Ben-Gurion University, Beer-Sheva*

³*Brull Community Mental Health Center, Tel-Aviv University*

Introduction: Antidepressants were found to facilitate neuroplasticity and cognition by stimulating trophic factors. Insulin-like-growth-factor-1 (IGF-1) is a potent neurotrophic factor in the brain. Previous studies have demonstrated that IGF-1 accelerates brain growth and neuroplasticity. IGF-1 is regulated by different neurotransmitters such as norepinephrine (NE) and serotonin (5-HT).

Our aim was to evaluate the effect of selected antidepressants, which act differently on 5-HT and on NE neurotransmitters, on the IGF-1 system in different regions of the rat brain and to assess the effect of IGF-1 on cognition.

Patients / Methods: Frontal cortex and hippocampus were dissected from male Wistar rats treated with reboxetine and fluoxetine given orally (15mg/kg daily for 3 or 21 days). IGF-1 receptor (IGF-1R) expression was determined by Western blot analysis. IGF-1 mRNA levels were assessed by semiquantitative PCR reaction. To determine the role of IGF-1 in cognition, rats were injected with IGF-1 (5mcg/rat icv) and subjected to the Morris Water Maze (MWM) and to the object recognition task (OR).

Results: In the frontal cortex, both drugs decreased IGF-1 mRNA levels after 3d, and increased IGF-1 mRNA and receptor levels after 21d. In the hippocampus, reboxetine increased the receptor expression after 3d. Both drugs decreased IGF-1 mRNA levels, and reboxetine decreased also the receptor levels after 21d.

In the MWM, acute IGF-1 decreased the latencies to locate the platform. In the OR, the IGF-1 group tended to spend more time with the new object and showed significantly higher exploration activity in the arena compared to controls.

Conclusions: Our results suggest that IGF-1 possesses a short-term cognitive enhancing effect. Selected antidepressants affect IGF-1 system in the cortex and the hippocampus differently. After chronic treatment, the system is up-regulated in the frontal cortex, and tends to be down-regulated in the hippocampus. It is possible that antidepressants affect neuroplasticity through the IGF-1 system, and the frontal cortex might be one of the main regions involved.

Autocrine action of estrogen on the bovine mammary gland

Mr. Yonatan Feuermann¹ Dr. Sameer, Sameer Mabjeesh² Dr. Avi Shamay¹

¹*Institute of Animal Science, Agricultural Research Organization, the Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.*

²*Department of Animal Science, Faculty of Agriculture, Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel.*

Introduction: Leptin, a protein hormone produced and secreted predominantly by white adipose tissue, has a critical role in the regulation and coordination of energy metabolism. The identification of leptin in the milk of several mammals, including humans, led us to investigate its presence and regulatory effect in the cow mammary gland. The role of mammary fat pad and mammary adipocytes at the initiation of lactation is not clear. Prolactin release during parturition had been demonstrated in the past to be insignificant for the bovine lactogenesis. Yet, we have previously demonstrated the ability of prolactin to modulate the magnitude of lactation in the presence of leptin and we showed that prolactin up regulates leptin secretion from mammary fat. Since estrogen is one of the endo/paracrine factors that are synthesized and secreted from the mature adipocytes, we study whether mammary derived estrogen is involved in the regulation of lactation, and whether its action is affected by the interaction between leptin and prolactin.

Patients / Methods: Mammary tissue was obtained from cows in the slaughterhouse. Explants/primary culture were prepared and cultured at 37°C, 5% CO₂ in medium M-199/DMEM-F12 supplemented with Insulin or with Insulin, Prolactin and/or leptin. Primary culture was incubated with or without fat explants and different concentration of prolactin. At the end of the experiments, the Explants and medium were harvested and analyzed.

Results: In this study, we show that prolactin interacts with leptin to regulate estrogen actions in the bovine mammary gland. We show that leptin affects the expression of estrogen receptor alpha in the bovine mammary epithelial cells. On the other hand, prolactin affects StAR mRNA expression in the bovine mammary fat. We also show the ability of prolactin and leptin to up regulate ABCG2 expression (ABCG2 gene include the ERE) in mammary explants. The same effect was observed in cholesterol secretion from explants culture to the medium.

Conclusions: In this study we demonstrated that prolactin initiates estrogen expression (as represented by StAR and p450 mRNA) in the mammary fat pad and that leptin affects estrogen receptor alpha in the mammary epithelial cells. Since leptin secretion levels is directly related to adipocytes quantity or size, the amount or size of the mammary fat can regulate the sensitivity of the epithelial cell in the mammary gland to estrogen. We hypothesize that leptin and estrogen which are secreted from the mammary fat, can regulate lactation after stimulation of prolactin.

Abstract Code: P65

Effects of selective somatostatin analogs and cortistatin on cell viability in cultured human non-functioning pituitary adenomas

Dr. Hagit Padova Dr. Hadara Rubinfeld Prof. Moshe Hadani Dr. Ilan Shimon

*Institute of Endocrinology, Felsenstein Medical Research Center, Beilinson Hospital, Petah-Tiqva and Tel-Aviv University;
Department of Neurosurgery, Sheba Medical Center, Tel-Hashomer*

Introduction: Clinically “non-functioning” human pituitary adenomas (NFA) constitute about 35% of pituitary adenomas. Somatostatin receptors (SSTR) expression in these adenomas has previously been described both in vivo and in vitro, without evidence of a correlation with tumor volume or the therapeutic efficacy of somatostatin analogs. Cortistatin (CST) is a recently described neuropeptide, which shares high homology with somatostatin and binds with high affinity to all SSTR subtypes.

Patients / Methods: This study was performed on 13 surgically removed pituitary macroadenomas, diagnosed before surgery as “non-functioning”. In addition, 3 growth hormone (GH)-secreting adenomas served as controls. A specimen of each tumor was dispersed and digested to isolate and culture the tumor cells, and the in vitro effects of SSTR2 and SSTR5 selective analogs and CST (100 nM) on cell viability were studied. The quantity of viable cells was estimated using the XTT method. RNA purification of tumor samples and subsequent RT-PCR studies for SSTR2 and SSTR5 expression were performed.

Results: The somatostatin analog with high affinity for SSTR2 reduced cell viability by 20-50% in 9 of 13 NFAs, all expressing the SSTR2. In 10 of 13 NFAs studied, all but two expressing SSTR5, the inhibitory effect on cell viability of SSTR5-selective analog was 15-50%. CST, however, effectively reduced cell viability in only 6 NFAs. In 2 out of 3 GH-secreting adenomas expressing both receptors, cell viability was inhibited by all peptides examined. The third adenoma responded to SSTR2 analog and expressed only SSTR2.

Conclusions: These results suggest the involvement of SSTR2 and SSTR5 in the anti-proliferative effects of somatostatin. However, CST is less potent in reducing cell viability in these tumors.

Long-acting insulin analogues have mitogenic and antiapoptotic activities.

Doron Weinstein¹ Prof. Zvi Laron² Prof. Haim Werner¹

¹*Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv*

²*Endocrine and Diabetes Research Unit, Schneider Children's Hospital, Petah Tikva*

Introduction: Long acting insulin analogues have been developed to improve diabetes care. These analogues have modifications in the C-terminal regions of the alpha and beta chains of human insulin, which do not participate in mediating insulin binding to its receptor. However, these regions have an important role in determining ligand affinity towards the IGF-I receptor (IGF-IR), a transmembrane receptor with important roles in tumor biology. Our aim was to test whether Glargine (G1, Lantus®, Sanofi Aventis) and Detemir (Dt, Levemir®, Novo Nordisk), two new long-acting insulin analogues exhibit IGF-I-like activities, including enhanced mitogenic and antiapoptotic effects.

Patients / Methods: Colon (HCT116), prostate (PC3) and breast (MCF7) cancer-derived cell lines were incubated with IGF-I, regular insulin (rINS), G1, or Dt for different time intervals and then harvested and counted with a hemocytometer. In addition, the potential antiapoptotic activities of the analogues were evaluated using an Annexin V/FITC kit and the activated signaling cascades were identified by Western immunoblotting.

Results: Results obtained showed that both G1 and Dt significantly ($p < 0.05$) stimulated cell proliferation in HCT116, PC3, and MCF-7 cell lines. Thus, in HCT116 cells G1 and Dt induced a ~22% and ~18% increase in proliferation, respectively, and IGF-I 35%. rINS, on the other hand, had a negligible effect (~1%). In PC3 cells, G1 and Dt stimulated proliferation by ~10% and ~13%, respectively, and IGF-I by ~18%. In contrast, rINS had essentially no effect (~1%). A similar proliferative potential was exhibited by G1 and Dt in MCF-7 cells. Apoptosis measurements in HCT116 cells demonstrated that G1 and Dt exhibited an antiapoptotic effect after 12 h, similar to that elicited by IGF-I. Specifically, the percentage of apoptotic cells after G1, Dt, or IGF-I treatment were 14.9%, 18.1%, and 16.9%, respectively, whereas the portion of apoptotic cells after rINS or vehicle (control) treatment were 24.5% and 23.2%, respectively. Western blot analysis revealed that G1 activates both the MAPK and PI3K pathways, the major signaling cascades of both the insulin and IGF-I receptors.

Interestingly, the effect of G1 on the phosphorylation of AKT was stronger than that of IGF-I. **Conclusions:** In conclusion, both long-acting insulin analogues, G1 and Dt, exhibit potent mitogenic and antiapoptotic activities. These activities significantly exceed the extent of the effects elicited by rINS and seem to resemble IGF-I actions. Our data supplements that reported by Eckardt et al. (Diabetes 2006, Suppl. 1, Abst 463-P). The clinical implications of these findings remain to be established.

The role of cytosolic isocitrate dehydrogenase in the regulation of insulin secretion and biosynthesis

Veronique Attali¹ Prof. Nurit Kaiser¹ Dr. Gil Leibowitz¹

¹*Endocrinology and Metabolism Service, Department of Internal Medicine, Hadassah-Hebrew University Medical Center, Jerusalem*

Introduction: The cellular redox state is an important metabolic variable which influences many cellular functions. In the pancreatic β -cells, the redox state correlates with insulin secretion. It has been suggested that NADPH serves as a signal that couples glucose stimulation to insulin secretion. In β -cells, NADPH is the by-product of cytosolic isocitrate dehydrogenase (cIDH) activity. In addition, the oxidation of cytosolic isocitrate to α -ketoglutarate (aKG) via the NADP-dependent cIDH followed by re-entry of aKG into the mitochondria supplies fuel to the TCA cycle. Our aim is to study the role of cIDH in β -cell adaptation to insulin resistance and to test the hypothesis that over-expression of cIDH can protect β -cells from the deleterious effects of gluco-lipototoxicity.

Patients / Methods: The effects of cIDH over-expression on β -cell function were studied in the INS-1E β -cell line and in islets of Psammomys obesus (P. obesus), an insulin-resistant animal that serves as model for Type 2 diabetes. cIDH was expressed in islets derived from prediabetic, P. obesus and in INS-1E β -cells using an adenoviral vector. We studied the effects of cIDH over-expression on insulin secretion and proinsulin biosynthesis under normoglycemic conditions and following chronic exposure to high glucose and free fatty acids (FFAs), using an in vitro culture system.

Results: cIDH over-expression in P.obesus islets induced ~50-fold increase of cIDH enzyme activity, accompanied by 1.5-2 fold increase of insulin secretion in response to 16.7mM glucose, without affecting basal insulin secretion. In contrast, glucose-stimulated proinsulin biosynthesis was not modified. Chronic exposure of P. obesus islets and of INS-1E β -cells to high glucose and elevated FFA markedly inhibited glucose-stimulated insulin secretion and decreased islet insulin content. Over-expression of cIDH improved glucose-stimulated insulin secretion under conditions of gluco-lipototoxicity in P. obesus islets. Preliminary results in INS-1E β -cells show that cIDH over-expression attenuated the deleterious effect of gluco-lipototoxicity on insulin secretion but not on insulin content.

Conclusions: Augmentation of cIDH activity improves glucose-stimulated insulin secretion, but not proinsulin biosynthesis, in β -cells exposed to high glucose and FFAs. Therefore, cIDH may be a therapeutic target for amplifying insulin secretion and for protecting pancreatic islets from the deleterious effects of gluco-lipototoxicity in Type 2 diabetes.

Interference of GLUT4 gene expression by siRNA impairs IGF-I regulated skeletal growth - A potential mechanism for type 1 diabetes associated growth defect

Dr. Gila Maor¹ Ms. Roni Hazan Bril^{1,2} Prof. Eddi Karnieli²

¹*Anatomy and cell Biology, Faculty of Medicine, Technion*

²*Endocrinology, Diabetes and Metabolism, Rambam Medical Center and Faculty of Medicine – Technion, Haifa, ISRAEL*

Introduction: Introduction: Uncontrolled type 1 diabetic (DM1) patients frequently have impaired skeletal growth that improves upon insulin therapy. However the molecular mechanism is yet unclear. In skeletal growth centers (GC) glucose transporter 4 (GLUT4) and IGF-I receptor (IGF-IR) are co-regulated. Further, in murine mandibular condyle (MC) a model for endochondral ossification (EO), IGF-I modulates GLUT4 expression and function via IGF-IR signaling cascade. The purpose of the current study was to examine the impact of GLUT4 modulation on IGF-I-induced skeletal growth.

Patients / Methods: Methods: The experimental model was mandibular condyle-derived cells (MCDC) which follow EO cascade. Cells were treated with either insulin or IGF-I and effects on proliferation and differentiation (e.g. DNA synthesis, collagen and aggrecan synthesis) were monitored, as well as uptake of 2DOG for monitoring GLUT4 activity. To determine the role of GLUT4 in IGF-I activity, GLUT4 expression was shut down using RNA silencing approach, and its effects on IGF-I and insulin treated cultures were studied.

Results: Results: Mandibular condyle-derived cells (MCDC) were preincubated with either insulin or IGF-I for up to 12 days. Both insulin and IGF-I enhanced proliferation and differentiation (e.g. DNA synthesis, collagen and aggrecan synthesis) and EO process concomitant with increase in GLUT4 gene expression. Compared to basal state, insulin and IGF-I pre-incubation increased 2-deoxyglucose uptake by 32% and 125% above the basal level respectively, resulting from GLUT4 translocation to the outer-cellular membrane (as assessed by immunohistochemistry and confocal microscope). To determine the role of GLUT4 in IGF-I effect on bone growth, we used siRNA technique. GLUT4 gene expression was shut down by 56% after 48 hr and gradually recovered thereafter. This abolished IGF-I induced effects on both proliferation and differentiation. Surprisingly, IGF-I failed to stimulate aggrecan synthesis up to 48 hr after GLUT4 levels were fully recovered. However, insulin-induced growth, was only slightly affected by GLUT4 gene silencing.

Conclusions: Conclusions: Our data emphasizes the important role of GLUT4 in IGF-I induced skeletal growth. Thus, even temporary reduction in bone GLUT4 content, as probably occurs in uncontrolled DM1 patients, is “imprinted” in the “cell memory”, leading to potential long term impairments in skeletal growth.

Insulin-like mechanism of Glucose Tolerance Factor (GTF)

Dr. Tal Mizrahi¹ Dr. Nitsa Mirsky¹

¹*Haifa University*

Introduction: The Glucose Tolerance factor (GTF) is a dietary agent derived from yeast. It is not a protein and can be administered orally. Although it has not been fully identified yet, the partially purified material remarkably lowered blood glucose and lipids in diabetic animals, and decreased peroxidation processes in their organs. When combined with low doses of insulin, GTF potentiated insulin action in the treated animals. In vitro studies done in our laboratory on various cell lines showed insulin like effects for GTF: GTF increased glucose transport into the cells. When a combination of GTF and insulin was supplemented to the cells, a synergy between GTF and insulin was detected. We hypothesize that GTF exerts its insulin-like effects via the insulin signaling pathway.

Patients / Methods: GTF was extracted and partially purified from yeast according to a method published by our laboratory. Differentiated 3T3-L1 adipocytes were serum starved for 18 hr, cells were treated with either 100nM insulin or with 20 mg/ml GTF for the indicated time. Cells were lysed, and western blot analysis was performed with anti-phospho antibodies followed by stripping and reblotting with antibodies for total proteins.

Results: Treatment of 3T3-L1 adipocytes with GTF increased general tyrosine phosphorylation of proteins in a time and dose-dependent manner. Whereas GTF did not affect tyrosine phosphorylation of insulin receptor (IR), it increased tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and stimulated the activation of Akt and p44/42 MAPK, two downstream key proteins in insulin signaling pathway. Activation of Akt by GTF was inhibited by both wortmannin and LY294002, inhibitors directed against phosphatidylinositol-3-kinase (PI3K). The selective tyrosine kinase inhibitor HNMPA-(AM)3 blocked GTF-induced phosphorylation of tyrosine, Akt and MAPK, suggesting that GTF exerts its effects by activating upstream tyrosine kinases.

Conclusions: Our findings demonstrate that GTF acts through the insulin-signaling pathway, and indicate a potential future use of GTF as an "insulin-like" material.

Abstract Code: P70

Dynamics of Cell Cycle Machinery in Pancreatic Beta Cells

Mr. Seth Salpeter¹ Dr. Tzvika Granot¹ Dr. Yuval Dor¹

¹*The Hebrew University-Hadassah Medical School Jerusalem*

Introduction: Both Type I and Type II diabetes stem from a deficiency in beta cell mass. Understanding the machinery and dynamics of beta cell replication may provide a key insight into our ability to increase beta cell number both in vivo and in vitro. Though several important factors in the beta cell cycle have been identified, the process that leads to cell cycle entry and division remains unknown.

Patients / Methods: To gain insight into the molecular mechanisms of beta cell proliferation, we have started to map the in vivo expression of genes involved in beta cell proliferation, using confocal microscopy

Results: We show that throughout the life span of the mouse, almost all beta cells – including both proliferating and quiescent cells- possess a nuclear CDK4 and Cyclin D2, two proteins thought to be necessary and sufficient for cell cycle entry. However, the CDK4-CyclinD2 kinase complex appears to be inactive in resting cells, as its key substrate, the retinoblastoma protein, becomes phosphorylated only in proliferating beta cells.

Conclusions: Therefore, in contrast to standard cell cycle models, the proliferation of beta cells is controlled downstream of CDK4 and CyclinD2 nuclear accumulation. Further experiments are underway to characterize the signal that activates the CDK4-CyclinD2 complex to regulate beta cell proliferation.

Cystic fibrosis-related diabetes: the role of peripheral insulin resistance and beta-cell dysfunction

Dr. Dalit Modan-Moses¹ Dr. Orit Pinhas-Hamiel¹ Dr. Kineret Mazor¹ Ms. **Taipora Ziv**¹ Dr. Asher Barak² Dr. Yaakov Yahav² Dr. Ori Efrati²

¹*Pediatric Endocrinology Unit, The Edmond and Lily Safra Children's Hospital, Tel-Hashomer*

²*Pediatric Pulmonology Unit, The Edmond and Lily Safra Children's Hospital, Tel-Hashomer*

Introduction: Cystic fibrosis (CF) is the most common lethal autosomal recessive disease in whites, affecting 1 in 2,500 births. Cystic fibrosis-related diabetes (CFRD) occurs in 30–40% of adults and is associated with increased morbidity and mortality, accelerated decline in lung function, decreased BMI, and increased likelihood of infection with *Pseudomonas* species. Insulin deficiency is an essential factor in the development of CFRD. An additional contribution of insulin resistance (IR) has been reported, but the role of IR in the pathogenesis of CFRD remains unclear.

Our study aim was to investigate the roles of peripheral insulin resistance and pancreatic -cell dysfunction in the pathogenesis of CFRD in CF patients with no previous history of glycemic disturbances.

Patients / Methods: Thirty-nine CF patients underwent 3-h oral glucose tolerance tests (OGTTs). Peripheral IR was measured using the homeostasis model assessment for insulin resistance (HOMA-IR). Pancreatic-cell function was calculated as the ratio between the 30-min increment in plasma insulin and the corresponding 30-min post-OGTT plasma glucose concentration.

Results: Of the 39 CF patients studied, 6, 26, and 7 were found to have normal, impaired and diabetic glucose tolerances, respectively. HOMA-IR was inversely correlated with fasting glucose ($r=0.44$, $p=0.008$). The mean HOMA-IR values of diabetic patients was significantly ($p=0.03$) increased compared with the patients with impaired or normal glucose tolerance (1.94 ± 0.69 , 1.03 ± 1.1 , and 0.99 ± 0.4 mU/mmol, respectively). Similarly, beta-cell function was significantly ($p=0.03$) lower in the diabetic group compared with the patients with impaired or normal glucose tolerance (0.7 ± 0.54 , 1.44 ± 0.91 , and 2.16 ± 1.05 mU/mmol, respectively).

Conclusions: Our results suggest that both peripheral IR and pancreatic beta-cell dysfunction with insulinopenia play a role in the pathogenesis of CFRD.

Phosphodiesterase Isoenzymes As Target For Enhanced Insulin Response In Pancreatic Beta-Cells

Ms. Avital Dov¹ Mr. Nasim Warwar¹ Mrs. Eva Abramovitch¹ Dr. Rafael Neshet¹

¹*Endocrinology and Metabolism Service, Department of Medicine, Hadassah - The Hebrew University Medical Center, Jerusalem*

Introduction: Multiple potentiating signals are known to serve as amplifying elements originating from glucose metabolism or from hormonal and neuronal receptors in pancreatic beta cells, including, ATP, lipid messengers, cyclic nucleotides and calcium. Cyclic AMP (cAMP)-dependent pathways are the site of convergence of many regulatory stimuli and cAMP is the most important potentiator of insulin response to glucose. Cyclic AMP is degraded by a family of enzymes known as cAMP-phosphodiesterase (PDE), which is the only known system for turning-off the nucleotide's signal. There are 11 known PDE gene families, each containing multiple genes, alternate promoter initiation site and/or alternate splicing of mRNA, yielding more than 50 isoenzyme variants. Here we identified all PDE isoforms expressed in rat beta-cell and rat islets. Furthermore, we examined the effect of shutting-off PDE3A and PDE8B isoenzymes on the kinetics of insulin secretion in pancreatic beta-cells

Patients / Methods: Rat pancreatic beta-cell PDE isoenzymes were identified by rt-PCR and by western blot analysis in INS-1E cells and in isolated rat islets. RNAi constructs were used to diminish PDE3A and PDE8B levels. The function of PDE3A and PDE8B in response to glucose stimulus was examined in batch incubations or in perfused islets

Results: PDE3A, PDE3B, PDE4B and PDE8B were identified at the mRNA and protein level in INS1E cells and in rat islets. Diminished PDE3A levels enhanced insulin response to glucose in INS-1E cells and had no effect on rates of cell replication. Two splice variants of PDE8B were identified in rat beta-cells: a 70 kDa and an 85 kDa isoforms. Diminished PDE8B levels significantly enhanced insulin response to glucose in INS-1E cells.

Conclusions: This is the first report identifying PDE8B in pancreatic beta-cells. Our data suggests a role for PDE3A and PDE8B in the potentiation of beta-cell insulin response to glucose. Since cAMP has multiple regulatory functions in pancreatic beta-cells, the site of action and the functional relationship between PDE3A and PDE8B in beta-cells are of considerable interest. Elucidation of the specific function of each of the four isoforms of PDE found in pancreatic beta-cell may help to identify a novel site to improve insulin response in type 2 diabetes mellitus.

Characterization of Atherosclerotic Lesions in apoE-deficient mice with Scanning Electron Microscopy (SEM) of Wet Tissue

Dr. Hofit Cohen¹ Dr. Aviv Shaish¹ Dr. Yehuda Kamari¹ Ms. Anya Vainshtein²
Dr. Rafael Bizur¹ Dr. Arnon Afek Ms. Shlomzion Shen² Dr. Dror Harats¹

¹*The Institute of Lipid and Atherosclerosis Research, Sheba Medical Center, Tel-Hashomer and Sackler Faculty of Medicine Tel Aviv University, Israel*

²*Quantomix Ltd., Rehovot, Israel*

Introduction: Premature atherosclerosis in the diabetic patients' population is accountable to their greater risk of cardiovascular morbidity and mortality. Development of therapies for atherosclerosis is dependent upon improved assessment of atherosclerotic lesion in animal models. We present a novel technique utilizing scanning electron microscopy (SEM) for imaging wet biological specimens

Patients / Methods: ApoE-deficient mice, fed an atherogenic diet served as the model of atherosclerosis. Samples of their aortas were placed in the QX-302 capsule for wet SEM imaging.

Results: Morphological characterization of the early and advanced atherosclerotic lesions was carried out and quantitative demonstration of the lipid-rich lesion was done with osmium tetroxide staining for cholesterol. Gold immunolabeling of specific epitopes was applied in the identification of the cellular and molecular components within the atherosclerotic lesions- namely foam cells, smooth muscle cells and collagen.

Conclusions: The study in the Wet SEM technique demonstrates an accurate and detailed structural evaluation of the process of atherosclerosis. Comprehension of the diabetes associated mechanisms that precipitate the atherosclerotic process, utilizing the technique, may assist in the emergent of innovative therapeutic intervention of CVD management and prevention in diabetes.

Prevalence of dyslipidemia and other cardiovascular risk factors in obese children and adolescents referred to a tertiary care center in Israel

Dr. shlomit shalitin^{1,2} Prof. moshe phillip^{1,2}

¹*Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel, Petach Tikva*

²*Sackler Faculty of Medicine, Tel Aviv*

Introduction: Childhood obesity is considered a worldwide epidemic, with increased prevalence rates. Of the various consequences of obesity in childhood, dyslipidemia was reported to be the most common. The aim of our study was to establish the prevalence of dyslipidemia in Israeli obese children and adolescents and to examine its association with anthropometric and metabolic risk factors of cardiovascular disease (CVD).

Patients / Methods: A retrospective design was used. The files of 262 obese children and adolescents [body mass index (BMI) over 95th percentile for age and gender] referred to a tertiary center in Israel were reviewed for anthropometric, clinical, and biochemical parameters of CVD risk (age 5.1-18.8 years). Patients with dyslipidemia defined as cut off point >95th percentile of healthy children (total cholesterol >200 mg/dL, triglycerides >150 mg/dL, LDL cholesterol >130 mg/dL, or HDL cholesterol <35 mg/dL) were compared with patients with normal values.

Results: Seventy-one patients (43 of 142 females, 30.3% and 28 of 120 males, 23.3%) had dyslipidemia. Compared to the patients with a normal lipid profile, the dyslipidemic patients had a significantly higher prevalence of fatty liver ($p < 0.001$), higher percent body fat in males ($p = 0.044$), and higher C-reactive protein (CRP) levels in females ($p = 0.001$). There was no significant difference in age, ethnicity, presence of hypertension or acanthosis nigricans, Tanner stage, BMI-standard deviation score (BMI- SDS), insulin resistance (HOMA-IR), or family history of diabetes or CVD-related disorders. Hypertension was found in 137 patients (75 females, 52.8% and 62 males, 51.7 %). This group was significantly older than the normotensive patients ($p < 0.001$) and had a higher percent of body fat ($p = 0.008$), larger waist circumference ($p < 0.001$), and a higher HOMA-IR ($p < 0.001$). No significant difference was noted between the groups in BMI- SDS, CRP level, or family history of CVD-related disorders.

Clustering was noted for the risk factors of dyslipidemia, hypertension, and HOMA-IR >2.5: 32.1% of the patients had 1 risk factor, 36.7% had 2, and 14.6% had 3. On multiple regression analysis, the most significant factors associated with CVD risk were waist circumference and insulin level.

Conclusions: Obese Israeli children and adolescents have high rates of CVD risk factors. Since most of them were unrelated to degree of overweight, gender, or age, we recommend screening for all of them in obese children of any age to detect those who require more intense interventions.

Apparent Role of 3T3-L1 12-Lipoxygenase in preventing adipocyte apoptosis and facilitating fat accumulation

Dr. Rona Limor¹ Anat Revzin¹ Dr. Gary Weisinger¹ Prof. Naftali Stern¹

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

Introduction: Weight gain and obesity are characterized by fat mass expansion resulting from adipocyte hypertrophy and enhanced lipid accumulation, some of which is shunted to rapidly differentiating committed pre-adipocytes that heavily populate fat tissue. Because our earlier work indicated that the platelet type 12 lipoxygenase (12-LO) has anti-apoptotic effects in vascular smooth muscle cells, we explored its role in adipocyte survival using the 3T3-L1 cell line of murine pre-adipocytes.

Patients / Methods: The cell line was cultured and differentiated using standard methods. Cell death was illustrated by Trypan Blue staining, LDH release and histone-DNA fragment detection. The cell differentiation was demonstrated by oil red staining and the expression of differentiation markers was measured by RT-PCR method

Results: We found that both mouse platelet type 12LO and the mouse epidermal type 12-LO are expressed in 3T3-L1 cells, but 12-LO expression was higher in pre-adipocytes than in differentiated adipocytes. Biochemical inhibition of lipoxygenase enzymes by two different inhibitors, esculetin (10-6M) and baicalein (10-6M) resulted in increased 3T3-L1 cell death in both the pre-adipocyte and the differentiated adipocyte state. In differentiated 3T3-L1, cell death was not necrotic as evidenced by lack of rise in the release of LDH to the culture medium, but rather associated with the release of histone-DNA fragments (Roche Elisa detection assay), thus indicating an apoptotic process. Additionally, baicalein treatment (10-6M) diminished the rate of fat accumulation in differentiating 3T3-L1 cells, assessed by oil red staining, but had no effect on the mRNA profile over time of several genes such as PPAR-gamma-2, and adipocyte fatty acid binding protein-2 (ap-2) whose expression is dynamically altered during the differentiation process. When 12 hydroxyeicosatetraenoic acid (12-HETE) (10-7 M, every 3 days) was added to baicalein-treated differentiated 3T3-L1, cell death could be fully inhibited and the rate of fat storage was partially restored

Conclusions: 12-LO is expressed in murine 3T3-L1 pre-adipocytes as well as in differentiated adipocytes, in which its arachidonate product 12 HETE prevents apoptosis and facilitates fat accumulation, in the absence of any effect on several genes linked to cell differentiation. The precise mechanisms by which 12-HETE's anti-apoptotic effect in adipose cells is exerted require elucidation. Whether or not 12-LO inhibition can be utilized to reduce fat cell population and decelerate fat storage in obesity is the subject of ongoing studies.

Oncogenesis in mammary epithelial cells of transgenic mice expressing Stat5 variants: conditions for cancer development and characterization of gene expression profiles in tumors

Mrs. Tali Eilon¹ Dr. Itamar Barash¹

¹*The Volcani Center. Institute of Animal Science. Bet-Dagan*

Introduction: The signal transducer and activator of transcription (Stat5) funnels extracellular signals into transcriptional activity. Our group established its oncogenic role in the mammary gland. Here, we studied the physiological requirements for epithelial cell transformation by deregulated Stat5 and characterized specific gene expression profiles in the developing tumors.

Patients / Methods: In transgenic mice we studied the role of Stat5 deregulation on mammary tumorigenesis.

Results: Tumors induced by overexpression of the native Stat5 (STAT5) or its forced-activated variant (STAT5ca) were detected in the mammary glands of multiparous transgenic female mice, but rarely in their aged-matched virgin (AMV) counterparts. The average latency period of 14 months which was required for tumor development exceeded the fertile cycles, and was independent of subsequent tumor-phenotype or transgenic Stat5 variant effects. No differences in endogenous and transgenic Stat5 levels or activities could be found between menopausal multiparous and AMV females. In contrast, in menopausal multiparous females expressing STAT5ca, a more relaxed chromatin pattern encompassed the Stat5-binding sites in the promoters of the cyclin D1 and bcl-x genes. Consequently, a small subset of epithelial cells overexpressing both phosphorylated Stat5 and cyclin D1 was defined exclusively in the multiparous glands.

Overexpression and forced activation of Stat5 caused tumors of a differentiated phenotype, mainly adenocarcinoma. In contrast, overexpression of a Stat5 variant lacking its transactivation domain resulted in poorly differentiated carcinoma. By array analysis we have compared gene expression in the differentiated vs. non differentiated tumors and showed specific clusters of genes, assembled to metabolic pathways which characterize each phenotype. For example, high expression of the canonical WNT pathway characterizes the papillary adenocarcinoma, while members of the integrin family, their respective ligands and downstream signaling molecules were highly expressed in carcinoma.

Conclusions: We suggest that the more relaxed chromatin pattern in the multiparous mice enables better accessibility of the deregulated Stat5 and amplifies its effect, resulting in overexpression of cyclin D1 and tumorigenesis in mammary epithelial cells.

A comprehensive analysis of gene expression profiles in the diverse tumor phenotypes contributes to our understanding of the forces mediating the appearance and the properties of the mammary gland tumors.

Mechanisms for cancer prevention by dietary compounds: A role for the Electrophile/Antioxidant Response Element and the Nrf2 transcription factor

Mrs. Yaara Amosi¹ Mrs. Karin Linnewiel¹ Dr. Keren Hirsch¹ Mrs. Hagar Salman¹ Prof. Joseph Levy¹ Prof. Yoav Sharoni¹

¹*Department of Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University of the Negev and Soroka Medical Center of Kupat Holim, Beer-Sheva, Israel*

Introduction: Androgens are important risk factors for the hormone dependent prostate cancer. We previously demonstrated that carotenoids inhibit androgen-induced cancer cell growth (thymidine incorporation), androgen signaling (luciferase reporter gene assay) and expression of androgen dependent protein (PSA by IRMA). In addition we reported that carotenoids induce phase II enzymes by activation of the Electrophile/Antioxidant response element (EpRE/ARE) and the Nrf2 transcription factor. The aim of the current study was to determine whether other dietary active compounds such as the polyphenols carnosic acid and curcumin inhibit androgen signaling and if the Nrf2 transcription factor and the EpRE/ARE transcription system are involved in the inhibition of androgen signaling in hormone responsive prostate cancer cells.

Patients / Methods: .

Results: The carotenoid lycopene and the polyphenols carnosic acid and curcumin inhibited the androgen-induced reporter gene activity in LNCaP prostate cancer cells. Ectopic expression of Nrf2 or activation of endogenous Nrf2 by tert-butyl-hydroquinone (tBHQ, a classical activator of Nrf2) resulted in the inhibition of androgen-induced reporter genes activity similar to that achieved by incubating the cells with carotenoids and polyphenols. Using IRMA we found that lycopene, carnosic acid, tBHQ and Nrf2 over-expression inhibited the expression of the androgen-induced protein, PSA. Transient or stable transfection of LNCaP cells with DN-Nrf2, which prevented ARE activation by these micronutrient, only partially abrogate their inhibitory effect on androgen response element activity, suggesting that the inhibition of androgen activity by Nrf2 is not due only to EpRE/ARE activation. Therefore, we hypothesized that the inhibition of androgen signaling by at least some dietary compounds involves a direct interaction between Nrf2 and the androgen receptor transcription complex. This hypothesis is currently analyzed by transient and stable transfections with Nrf2-siRNA, which reduces Nrf2 level and EpRE/ARE activity.

Conclusions: Carotenoids and some polyphenols can prevent cancer by two mechanisms, both related to the activation of the Nrf2 transcription factor. Primarily the activated Nrf2 stimulates Phase II detoxifying enzymes by binding to EpRE/ARE sequences present in their promoters. In addition Nrf2 can interact directly with androgen receptor transcriptional complexes to inhibit the induction of multiple cancer-related genes such as PSA.

Affinity Targeting Of The Cytotoxic Drug Daunomycin With 7-(O)-Carboxymethyl Daidzein Improves The Therapeutic Response In Animal Model Of Human Ovarian Cancer

Dr. Fortune Kohen¹ Dr. S., S. Katzburg² Dr. N. Nevo³ Dr. R. P. Hodge⁴ Dr. M. D. Renevey⁵ Dr. V., V. Kalchenko⁶ Dr. N. Stern⁷ Dr.D. Somjen⁸

¹*Biological Regulation, Weizmann Institute of Science*

²*Veterinary Resources, Weizmann Institute of Science*

³*Biological Regulation, Weizmann Institute of Science*

⁴*Pharmacology & Toxicology, Texas University Medical Branch at Galveston, USA*

⁵*Pharmacology & Toxicology, Texas University Medical Branch at Galveston, USA*

⁶*Veterinary Resources, Weizmann Institute of Science, Veterinary Resources, Weizmann Institute of Science*

⁷*Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv University*

⁸*Institute of Endocrinology, Metabolism and Hypertension, Tel-Aviv University*

Introduction: Dose limitation of cytotoxic drugs often reflect the fact that the concentrations required to kill tumor cells are close to those that produce severe toxicity in normal cells. Affinity targeting of cytotoxic drugs with carboxy derivatives of isoflavones may overcome some of these drawbacks. In this study we explored the ability of the daunomycin conjugate of 7-(O)-carboxymethyl daidzein (cD-Dau) to act as an affinity carrier in vivo in nude mice using MLS ovarian xenografts.

Patients / Methods: cD was conjugated to the amino sugar residue of Dau to yield the cytotoxic conjugate cD-Dau. This conjugate was tested for its ability to inhibit DNA synthesis as assessed by ³[H] thymidine incorporation in MLS ovarian cancer cell line expressing a general abundance of ER α to ER β mRNA (ER α to ER β mRNA expression ratio= 30:1). The therapeutic efficiency of cD-Dau, Dau and vehicle) was tested in nude mice bearing MLS ovarian xenografts. The fluorescent signal due to Dau in the tumor was visualized on an IVIS 100 system.

Results: MLS cells were sensitive to estradiol-17 β and to genistein, daidzein, biochanin A as determined by induction of DNA synthesis and creatine kinase specific activity, which could be inhibited by the anti-estrogen ICI 172680, but only partially by raloxifene. cD-Dau inhibited DNA synthesis in a dose dependent manner. At low concentrations (0.3 to 3.0nM) the cytotoxic index of cD-Dau was 2 to 5 fold higher than Dau, but this difference was not persistent at higher concentrations (30 to 300nM). Mice bearing the MLS xenografts were injected i.v. every second day for ten days with cD-Dau (0.192 mg of Dau equivalent per injection) or Dau (0.2 mg/injection). Tumor growth in the groups of cD-Dau and Dau were inhibited by >50 % as compared to the tumor volume of untreated mice. No weight reduction was seen in mice treated with the cD- Dau conjugate, whereas Dau alone induced 15% reduction in mean body weight and caused death of 2 mice. While in vivo imaging of the fluorescence signal generated by Dau, with an IVIS system indicated uptake of both cD-Dau and Dau by the tumor, the intensity of fluorescence was higher in the the cD-Dau conjugate treated mice than in the Dau treated mice, thus suggesting specific delivery of the drug to the tumor.

Conclusions: These results indicate that targeting of Dau via cD markedly reduced its toxicity in an animal model of ovarian cancer. Utilization of the readily available membranal and /or nuclear estrogenic binding sites in these ovarian tumors for affinity targeting of daunomycin forms the basis for the broader use of cancer type-specific and hormone receptor specific therapy.

Hormonal regulation of hyaluronan synthase and its effect on the adhesion process of human epithelial ovarian carcinoma metastasis

Mrs. Yael Tzuman¹ Dr. Dorit Granot¹ Dr. Galit Mazooz¹ Dr. Nava Nevo¹ Prof. Michal Neeman¹

¹Weizmann Institute of Science, Rehovot

Introduction: Ovarian carcinoma is the leading cause of death among gynecological cancers, and strong data link transformation with ovulation. Nevertheless, the disease typically occurs in older patients, and is significantly more severe in postmenopausal woman. Among the changes that could induce progression of ovarian cancer during menopause are the elevation in gonadotropin levels, and the reduction in estrogen and progesterone levels. These changes in the hormonal environment may lead to initiation of ovarian cancer metastasis, which is manifested by multifocal seeding of cells or small spheroids on the mesothelial layer lining the peritoneum. This initial seeding is associated with adhesion onto hyaluronan (HA). Our goal is to investigate the effect of LH and FSH on HA synthase (HAS) levels in human ovarian carcinoma cells and in the mesothelium, as well as its effect on the adhesion process.

Patients / Methods: MLS cells were administered with increased levels of either LH or FSH (1ng/ml, 10ng/ml, 100ng/ml, and 500ng/ml) in varying time points (6h, 12h, 18h, 24h, and 48h). Total RNA was extracted from the cells, reversed transcribed, and semi-quantitative PCR analysis of HAS2 and HAS3 gene expression took place.

Aggregation of cells into large spheroids of ~1 mm was initiated by plating cells from a confluent culture onto agar-coated plates. 72 hours later, the spheroids were transferred into a 500 ml spinner flask and grown for ~1 month. 100µl of DiAsp fluorescently stained spheroids were i.p. injected into ovariectomized CD-1 nude mice. Adhesion of the spheroids was detected 9.5 and 24 hours later by intravital and fluorescence microscopy.

Results: MLS cells stimulated with FSH, showed a significant induction in expression levels of both HAS2 and HAS3, while LH stimulation lead to an inhibition in expression levels of HAS2, and an induction in expression levels of HAS3.

A number of fluorescently dyed spheroids were detected on the mesothelium of ovariectomized mice whereas the non-ovariectomized control mouse did not exhibit any spheroid adherence.

Conclusions: LH and FSH effect the expression levels of HAS in ovarian cancer cells. This change in the hormonal environment might consequently favor the adherence of ovarian cancer spheroids onto the peritoneum. The hormonal effect on HAS and on the adhesion process may help shed light on the change in aggressiveness of ovarian cancer observed during menopause.

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Synergistic antileukemic effects of vitamin D3 derivatives and rosemary polyphenols in a mouse acute myeloid leukemia model

Ms. Ayelet Shabtay¹ Mrs. Hagar Sharabani¹ Mr. Ze'ev Barvish¹ Prof. Joseph Levy¹ Prof. Yoav Sharoni¹ Prof. Michael Danilenko¹

¹*clinical biochemistry department, Ben Gurion university*

Introduction: 1,25-dihydroxyvitamin D3 (1,25D3) is a potential anticancer agent. However, it has a marked toxicity at pharmacologically active doses. Attempts to overcome this problem have recently focused on the synthesis of vitamin D analogs with lower calcemic effects. Alternatively, we have shown that in human myeloid leukemia cells the effects of low, non-toxic doses of 1,25D3 can be synergistically enhanced by plant polyphenols, e.g., carnosic acid from rosemary. The major aim of this study was to determine whether carnosic acid-rich rosemary extract and the low-calcemic 1,25D3 analog (19-nor-Gemini Ro27-5646) can cooperate in the antileukemic effect using a mouse systemic leukemia model in vivo.

Patients / Methods: Proliferation and viability of murine myelomonocytic leukemia cells (WEHI-3B D-) was determined by Coulter counting and trypan blue exclusion assay, respectively. Cell cycle progression was measured by DNA staining with propidium iodide and FACS analysis. Leukemia was induced by intravenous inoculation of WEHI-3B D- cells in Balb/c mice. Separate groups of mice were treated with (i) vehicle, (ii) Ro27-5646 (intraperitoneally, 3 times a week), (iii) rosemary extract (mixed with food), and (iv) their combinations.

Results: When added alone, 1,25D3, its analog Ro27-5646, carnosic acid and rosemary extract inhibited WEHI-3B D- cell proliferation in vitro. When combined at low concentrations, polyphenols and vitamin D derivatives markedly synergized in growth inhibition. This was accompanied by G1 cell cycle arrest, as observed both in unsynchronized cells and, more clearly, in cells stimulated with serum after synchronization in G1 phase by mimosine. No significant induction of apoptosis or cytotoxicity was observed under these conditions. Mice inoculated with WEHI-3B D- cells developed systemic leukemia, as was evidenced by the appearance of large number of blast-like cells in peripheral blood and bone marrow. Ro27-5646 and rosemary extract alone had slight effects while the combined treatment resulted in a substantial synergistic increase in the life span of leukemia-bearing mice (by 80%), as compared to untreated animals.

Conclusions: These results indicate that vitamin D3 derivatives and plant polyphenols cooperate in the anti-leukemic effect not only in cell culture but also in the animal model, without toxicity. Thus, these combinations may be used as an alternative to conventional cytotoxic chemotherapy of myeloid leukemias.

Abstract Code: P81

AHNAK - new mediator of Insulin action: Implications for GLUT4

Mrs. Dafna Ben-Yosef¹ Dr. Michal Armoni² Dr. Chava Harel² Prof. Eddy Karnieli²

¹*Technion Institute of Biotechnology*

²*Rambam Medical Center*

Introduction: High levels of free fatty acids like arachidonic acid (AA) repress GLUT4 gene expression in cardiac muscle (CM). We showed that in the H9C2 cardiomyotubes this effect is mediated via GLUT4 promoter (GLUT4-P) -222/-197 bp region, but the mediator remains elusive.

Patients / Methods: Thus, nuclear extracts from AA-treated H9C2 cells were subjected to GLUT4-P-biotin-avidin affinity column, followed by mass spectrometry. This analysis detected AHNAK/desmoyokin giant protein attached to GLUT4-P. AHNAK is a phosphoprotein that participates in AA-mediated cellular signaling in cardiac and skeletal muscle. Thus, we studied a role for AHNAK in AA-mediated GLUT4-P repression in vivo and in vitro.

Results: In-vivo studies: Using the streptozotocin-diabetic (STZ) rat, and quantitative RT-PCR analysis, we found that AHNAK mRNA levels in cardiac muscle were 3-fold increased in STZ and returned to control levels upon 8-day insulin therapy (8-IT). GLUT4 mRNA levels were 20% decreased in STZ, while returning to 109% in 8 IT. In contrast, AHNAK levels in adipocytes were 2.5-fold decreased in STZ, and failed to return to control levels upon to 8IT. GLUT4 mRNA levels were 80% decreased in STZ, while elevated to 200 % in 8 IT.

In vitro studies: AA-induced hyperlipidemia (HL) (200uM, 24-48hrs) decreased mRNA levels of AHNAK to 40% and 50% in H9C2 cardiomyotubes and PRA, respectively. Transient co transfection of either C-terminal, middle part or N-terminal of AHNAK resulted in 55-60% repressed GLUT4-P activity in PRA. 5' deletion analysis of GLUT4-P pointed at several regions, the deletion of which led to GLUT4-P de-repression. These regions may be potential cis-elements mediating AHNAK activity.

Conclusions: Thus, beyond its role as mediator of AA activities at cell surface, our data introduce AHNAK as a potential regulator of GLUT4 gene expression, acting in a tissue-specific manner. Therefore, AHNAK is a potential molecular target for type II Diabetes therapy.

Abstract Code: P82

Protein Kinase C Alpha And Protein Kinase C Delta Differentially Regulate Insulin Signaling in Hepatocytes

Ms. Tamar Brutman-Barazani¹ Ms. Shlomit, Shlomit Aga-Mizrachi¹ Ms. Miriam Horovitz-Fried¹ Prof. Chaya Brodie¹ Prof. Sanford Sampson¹

¹*Bar-Ilan University, Ramat-Gan*

Introduction: The liver is a major insulin-responsive tissue responsible for glucose regulation. The binding of insulin to its receptor (IR) induces a cascade of events leading to the phosphorylation of downstream signaling elements such as PKB and GSK3, leading to glycogen synthesis and glucose uptake. One important component of this cascade is the Protein Kinase C (PKC) family of serine-threonine kinases. Studies in our laboratories have shown that Protein Kinase C delta is essential for insulin-induced glucose transport in skeletal muscle. It was also shown that insulin rapidly stimulates activity and phosphorylation of PKC delta in both skeletal muscle and hepatocytes. Studies conducted on skeletal muscle have shown that PKC alpha may play a role as a constitutive negative regulator in the insulin signaling pathway. It was shown in human skeletal muscle that PKC alpha inhibits the insulin-induced phosphorylation of IRS1 and activation of PI3K. The purpose of this study was to investigate the role of PKC alpha in insulin signaling pathway in hepatocytes.

Patients / Methods: Studies were conducted on the AML-12 (Alpha Mouse Liver) cell line. We used adenovirus constructs of wild type (WT PKC alpha) to overexpress PKC alpha. PKC alpha was blocked both by expression of dominant negative (DN) isoforms and the use of a pharmacological inhibitor GO6976.

Results: Our recent results have shown that inhibition of PKC delta either by treatment with rottlerin, or suppression of PKC delta expression by transfection with siRNA, reduced both the activation of PKB and the phosphorylation of GSK3 induced by insulin. Interestingly the inhibition of PKC alpha neither by treatment with GO6976 or by overexpression of dominant negative PKC alpha increased activation of PKB, phosphorylation of GSK3, and insulin-induced glucose uptake. In contrast, overexpression of wild type PKC alpha induced a decrease in PKB and GSK3 phosphorylation, and a decrease in insulin-induced glucose uptake.

Conclusions: We conclude that PKC alpha acts as a constitutive negative regulator of the insulin signaling pathway whereas PKC delta acts as a positive regulator.

This work was supported by the Russell Berrie Foundation and D-Cure, Diabetes Care in Israel.

Crosstalk between cAMP and vitamin D in keratinocytes

Ms. Jennifer Romano^{1,2} Dr. Amiram Ravid^{1,3} Prof. Ruth Koren^{1,2}

¹*Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel-Aviv University*

²*Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel-Aviv University*

³*Department of Cellular and Developmental Biology, Sackler Faculty of Medicine, Tel-Aviv University*

Introduction: Calcitriol, the hormonal form of vitamin D, increases keratinocyte differentiation and exhibits a dual effect on their proliferation, inhibitory in inflamed and stimulatory in intact skin. The hormone also exerts an anti-inflammatory action on psoriatic skin lesions while eliciting mild inflammation on healthy skin. Since keratinocytes under stress and during inflammation are exposed to cAMP elevating agents, we aimed to examine the crosstalk between vitamin D at the level of keratinocyte proliferation, activation of extracellular regulated kinase (ERK) and induction of the transcription factor c-Fos.

Patients / Methods: The non-tumorigenic immortalized human HaCaT keratinocytes, cultured in the absence of exogenous growth factors or other biological effectors were exposed to calcitriol (48 hours). Exposing HaCaT cells to 8-bromo-cAMP together with the phosphodiesterase inhibitor isobutylmethylxanthine attained increased levels of intracellular cAMP. Proliferation was assessed by [³H]thymidine incorporation and the levels of dually-phosphorylated, activated ERK and c-Fos by western blotting.

Results: Incubation with calcitriol enhanced thymidine incorporation by 70%. Thymidine incorporation was markedly inhibited by the addition of the ERK pathway inhibitor U0126 indicating that DNA synthesis was dependent on the constitutive activity of the ERK cascade. Elevation of cAMP inhibited thymidine incorporation in a dose dependent manner and also inhibited the enhancement of thymidine incorporation by calcitriol. Elevation of cAMP brought about a transient increase in ERK activation (50% 4 minutes after addition of cAMP elevating agents). This elevation was not affected by preincubation with calcitriol. 8 hours following cAMP elevation we observed a marked decrease in the level of activated ERK (66% ± 6%) and this decrease was not observed in calcitriol pre-treated cells. Expression of c-Fos in HaCaT keratinocytes is dependent on ERK activity as examined by the addition U0126. Incubation with calcitriol markedly increased c-Fos level. cAMP as a single agent did not affect c-Fos level, but it increased the level of c-Fos induced by calcitriol.

Conclusions: The crosstalk between vitamin D and cAMP signaling pathways is manifested on HaCaT cell proliferation, ERK activation and c-Fos expression. Although ERK activation is known to regulate cell proliferation and c-Fos expression, the combined effects of vitamin D and cAMP on the latter phenomena cannot be attributed to their effects on ERK activation.

Preparation and Characterization of Recombinant Leptin from Pufferfish, *Takifugu rubripes*

Ms. Michal Yacobovitz¹ Ms. Gili Solomon¹ Dr. Berta Levavi-Sivan¹ Prof. Arieh Gertler¹

¹*Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem*

Introduction: Leptins are cytokines secreted by adipocytes and other cells and involved in regulation of metabolism and immune response. While mammalian leptins have been extensively investigated so far no leptins from fish were purified and directly tested.

Patients / Methods: Synthetic cDNA encoding leptin from Pufferfish, *Takifugu rubripes* (pfLEP), optimized for expression in *Escherichia coli* was prepared according to published sequence. The respective cDNA was then inserted into pMON expression vector and transformed into Mon 105 *E. coli* strain. The pfLEP expressed upon induction with nalidixic acid was found almost entirely in the insoluble inclusion bodies (IBs). The IBs were isolated, the proteins solubilized in 4.5 M urea, at pH 11.45 in presence of cysteine, refolded and purified to homogeneity (as shown by SDS-PAGE under reducing conditions) by anion-exchange chromatography on Q-Sepharose according to unique protocol developed for this protein yielding a mixture of monomers and dimers which were subsequently, separated on gel-filtration Superdex™75 preparative column.

Results: The overall yields varied between 50 to 100 mg from 5 liters of fermentation culture. Circular dichroism (CD) analyses revealed similarity of the purified pfLEP secondary structure to that of other mammalian leptins. The purified monomers and dimers showed a single band of molecular mass of ~15 kDa in SDS-PAGE in the presence of reducing agent, whereas the dimer showed one band of the molecular mass of ~30 kDa in SDS-PAGE in the absence of reducing agent, indicating that the dimer is formed by S-S bonds. The purified monomeric and dimeric pfLEPs were stable at 1 mg/ml sterile solution at pH 10, at 4°C; -20°C and as lyophilized powder for at least two months. In contrast to mammalian leptins the monomeric pfLEP did not form a 1:1 complex with chicken leptin-binding domain (chLBD), most likely due to low affinity. Both pfLEPs were biologically active in promoting proliferation in BAF/3 cells stably transfected with the long form of human leptin receptor, but their activity was 4-5 orders of magnitude lower than that of human leptin. The specificity of this activity was further evidenced by the fact that it was fully inhibited by human leptin antagonist.

Conclusions: This work is the first report on purification and partial characterization of leptin from any fish species.

Insulin Increases Nuclear PKC Delta in L6 Skeletal Muscle Cells

Ms. Miriam Horovitz-Fried¹ Ms. Tamar Brutman-Barazani¹ Prof. Sanford Sampson¹

¹*Faculty of Life Sciences, Bar-Ilan University*

Introduction: Protein kinase C (PKC) isoforms are involved in the transduction of a number of signals important for the regulation of cell growth, differentiation, apoptosis and many other functions. PKC proteins are believed to reside in the cytoplasm in an inactive state, and after phosphorylation, translocate to the plasma membrane or to membranes of cytoplasmic organelles to become fully activated. Evidence has accumulated that PKC isoforms may also have an important role in the nucleus. We recently showed that insulin induces a rapid increase in PKC delta RNA and protein. In this study we attempted to elucidate the mechanism of the insulin-induced increase in nuclear PKC delta.

Patients / Methods: Studies were conducted on the L6 skeletal muscle cell line. Nuclear extracts were prepared according to standard techniques. Purification of the nuclear preparation was verified by SEM and by Western blotting for tubulin. Standard procedures were used for Western blotting, immunostaining and 35-S methionine uptake.

Results: Inhibition of transcription and translation blocked the insulin-induced increase in nuclear PKC delta. Inhibition of nuclear import did not block the insulin-induced increase in nuclear PKC delta protein. Inhibition of protein export from the nucleus reduced the insulin-induced increase in PKC delta in the cytoplasm, and increased it in the nucleus. The PKC delta induced by insulin could not be removed from the surface of isolated nuclei by trypsin digestion. Insulin also induced incorporation of 35S-methionine into nuclear PKC delta protein. Neither other growth factors nor Rosiglitazone increased nuclear PKC delta protein.

Conclusions: This study suggests the possibility that PKC delta protein may be translated in the nuclei of skeletal muscle cells in response to insulin, closely time-linked to its transcription in the nucleus. This possible mechanism might explain the rapid increase in PKC delta protein in the nuclear fraction, which appears to be unique to insulin. The idea of rapid transcription and translation of proteins is highly unconventional and runs counter to current concepts of protein synthesis. However, this mechanism may be unique for insulin and PKC. Studies have shown that certain PKC isoforms, PKC delta in particular, play a key role in the initial steps of IR signaling and may participate in IR internalization and tyrosine phosphorylation. Thus, expression of PKC isoforms, such as PKC delta, may be regulated in a rapid manner at multiple levels to sustain these kinases at effective concentrations during the course of insulin action in insulin target tissues. One of these mechanisms might include rapid and closely linked nuclear transcription and translation. This work was supported by the Russell Berrie Foundation and D-Cure, Diabetes Care in Israel.

Abstract Code: P86

Novel roles for p38 and ERK1/2 in human ejaculated spermatozoa: regulation of flagellar motility, hyperactivation and acrosome reaction

Mr. Tal Almog¹ Mr. Nir Etkovitz² Prof. Haim Breitbart² Prof. Zvi Naor¹

¹*Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv 69978, Israel*

²*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, 52900, Israel*

Introduction: Mammalian spermatozoa must undergo a process termed capacitation as they swim through the female reproductive tract in order to fertilize the egg. Flagellar motility facilitates the progression of sperm in their designated path. Capacitated sperm exhibit hyperactivated motility and are more prone to undergo the acrosome reaction. ERK1/2 and p38 MAPK are known to be involved in cellular processes such as differentiation and proliferation.

Patients / Methods: Human semen (>50 mil sperm/ml) was obtained from healthy donors with normal sperm density, motility and morphology (according to the WHO guidelines of 1992), or from males attending the Infertility and IVF Unit, Assaf Harofeh Medical Center, Israel) after 3 days of sexual abstinence.

Results: Here we report for the first time the presence of p38 in ejaculated human spermatozoa. p38 and ERK1/2 are mainly localized to the sperm mid-piece and in patches along the tail. ERK1/2 is activated following PMA treatment in a PKC-dependent and Ca²⁺-independent manner. ERK1/2 and p38 play opposing roles in sperm flagellar motility and hyperactivation. We found that ERK1/2 stimulates, while p38 inhibits sperm motility and hyperactivation. Surprisingly, both ERK1/2 and p38 have a positive role in the induction of acrosome reaction by PMA and Ca²⁺ ionophore, A23187.

Conclusions: Our data shed new light on the roles of MAPK cascades in the regulation of sperm flagellar motility and hyperactivation, and open a new vista into the potential mechanisms of male infertility.

Are steroids obligatory mediators of LH/hCG triggered resumption of meiosis in mammals?

Mr. Shmulik Motola¹ Mrs. Malka Popliker¹ Prof. Alex Tsafiriri¹

¹*The Bernhard Zondek hormone research laboratory, Department of biological regulation, the Weizmann institute of science, Rehovot, Israel.*

Introduction: The essential role of steroids in the mediation of gonadotrophic stimulation of oocyte maturation in amphibians and fish is well established. Such role of steroids in the regulation of meiosis in mammals has not been confirmed until recently. A series of publications from the laboratory of Hammes presented evidence that steroids mediate LH action on resumption of meiosis in the mouse and the rat (2004-06). In these studies it was claimed that progesterone, estrogen or testosterone can stimulate resumption of meiosis and that this action of LH can be blocked by specific antagonists of each of these steroid receptors. Here we examine the suggested role of steroids in the mediation of LH-stimulated resumption of meiosis in the rat and the mouse.

Patients / Methods: In vitro cultures of rat and mouse follicle-enclosed oocytes (FEOs) and cumulus-enclosed oocytes (CEOs) were used. In FEOs, that mature only in response to gonadotropins or other stimuli, we tested the ability of steroids to trigger meiosis or whether steroid antagonists block LH action on meiosis. The spontaneous resumption of meiosis in CEOs was blocked by the purine hypoxanthine (Hx; 4mM), a mild inhibitor of phosphodiesterases. This model was used to test whether steroids overcome the inhibition of meiosis by Hx.

Results: The progesterone antagonists mifepristone (RU 486; 25-50 micro molar) and Org 31710 (1-10 micro molar) as well as the estrogen antagonist faslodex (25- 50 micro molar) did not prevent LH-triggered maturation of rat FEOs. In accordance, the progesterone agonist, promegestone (R5020; 250-1000nM) or estradiol (250-1000 nM) did not stimulate the resumption of meiosis in rat and mouse FEOs, and both (100-250 nM) did not overcome the Hx inhibition of meiosis in rat and mouse CEOs. By contrast, progesterone and promegestone induced the maturation of *Xenopus* oocytes.

Conclusions: Contrary to recent claims, we could not provide evidence that steroids mediate LH stimulation of meiosis in rodents. Thus, in stark contrast to lower vertebrates, steroids do not seem to serve as an obligatory signal by which the somatic cells of the follicle transfer the gonadotrophic stimulation of meiosis to the oocyte. We suggest that this may be associated with the evolution of hierarchical follicle growth in mammals and ovulation of one or a few oocytes each cycle and the resulting high steroid levels in the ovary. Such local abundance of steroids in the ovary presumably precludes their use in mammals as a signal for the resumption of meiosis.

Identification and Characterization of an Ovulation-Dependent Novel Gene

Mrs. Ketty Shkolnik¹ Dr. Shifra Ben-Dor² Mrs. Dalia Galiani¹ Dr. Nava Nevo¹
Dr. Ariel Hourvitz³ Prof. Nava Dekel¹

¹*Department of Biological Regulation, Weizmann Institute, Rehovot, Israel*

²*Department of Biological Services, Weizmann Institute of Science, Rehovot 76100, Israel*

³*IVF Unit, Department of Obstetrics and Gynecology, Chaim Sheba Medical Centre, Tel-Hashomer, Israel*

Introduction: The release of the female germ cell from the ovary, known as ovulation, is a key event in mammalian reproduction. This complex process is initiated by the luteinizing hormone (LH) surge and is controlled by the expression of specific genes. The present study was designed to identify and characterize an LH-dependent novel gene that was isolated by the suppression subtractive hybridization (SSH) method.

Patients / Methods: We have employed bioinformatics search tools in combination with Real-Time PCR (RTP) method and RACE technique to identify the full-length cDNA sequence of the selected ovulatory-dependent novel gene. Furthermore, tissue array and in-situ hybridization analyses were performed by using hCG / PMSG-primed sexually immature mice as our experimental model. To elucidate the signaling pathway that regulates the expression of our novel gene, large antral PMSG-primed murine follicles were incubated with different pharmacological agents and submitted to RTP analysis of the gene expression.

Results: The full-length sequence of the selected ovulatory-dependent C9 clone has revealed a new isoform of the Ncoa7/ERAP140 gene. This new isoform has a unique and highly conserved exon at the 5' and encodes for a protein with a unique N terminal sequence. Tissue expression profile of C9 in a variety of mouse tissues has demonstrated abundant hCG-dependent ovarian expression, whereas the highest expression of Ncoa7 was in the brain. Treatments of the large antral follicles with forskolin and to a lesser degree, PMA, stimulated C9 mRNA expression. The stimulatory effect of LH on C9 mRNA was reduced by treatment with specific inhibitors of PKA, MAPK kinase, and p38 kinase.

Conclusions: We have identified a new highly conserved ovulatory-dependent gene. We further found that the induction of C9 mRNA expression is mediated by the LH-induced activation of various intracellular signaling pathways. Expression of the C9 isoform was most abundant in the ovary after hCG treatment, indicating that C9 may have a specific function during ovulation.

Induction Of Heparanase By LH During The Ovulatory Process

Dr. Eyal Klipper¹ Mr. Ehud Tatz¹ Prof. Israel Vlodaysky² Prof. Uzi Moallem³
Prof. Dieter Schams⁴ Prof. David Wolfenson¹ Prof. Rina Meidan¹

¹*Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel*

²*Cancer and Vascular Biology Research Center, The Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel*

³*Department of Dairy Cattle, Institute of Animal Sciences, Volcani Center, P.O. Box 6, Bet-Dagan, Israel*

⁴*Institute of Physiology, Weihenstephan, Technical University of Munich, Freising-Weihenstephan, Germany*

Introduction: Ovulation is a complex process that is initiated by the LH surge. Coinciding with the termination of specific gene expression in mature follicles, LH induces a set of genes involved in ovulation. Extensive tissue remodeling accompanies follicular rupture and the transformation of a preovulatory follicle into a highly vascularized corpus luteum (CL).

Patients / Methods: In this study we first examined whether heparanase, an enzyme endoglycosidase that cleaves the heparan sulfate glycosaminoglycans from proteoglycan core proteins, is induced during these processes, and then studied the regulation of heparanase expression in bovine follicular cells. Granulosa cells (GCs) were retrieved from first-wave dominant follicles (day 6 of the cycle), and from follicles collected 24 and 42 hrs after prostaglandin F2a (PGF2a) injection.

Results: Heparanase mRNA expression in GC increased slightly 24 and 42h after PGF2a administration, as compared with cells collected on day 6. A marked, 11-fold increase in heparanase mRNA was observed 12h after i.m. administration of GnRH to the preovulatory follicles. In a second study, the temporal pattern of heparanase gene expression in folliculo-luteal transition was examined in whole follicles collected before and 4, 10, 20, 25, and 60h (early CL) after GnRH. Heparanase expression was low before GnRH and rose significantly but transiently 10 and 20h after GnRH to levels app. 10-fold higher. Just before ovulation (25h post GnRH) and in early CL, heparanase levels returned to basal values. Immunostaining of follicles revealed that the enzyme was more abundant in the GC layer than in the theca internal layer. To better understand the regulation of heparanase, we incubated GC with LH and RU-486 (progesterone receptor antagonist). LH induced a transient increase in heparanase mRNA 3 and 6h after its addition. In contrast, the presence of RU-486 stimulated heparanase after 24h. During in vitro luteinization of GC, the levels of VEGF and StAR rose steadily, as expected, from day 2 to day 6 in culture, whereas heparanase mRNA remained unaffected in these luteal cells. Similarly, when challenged with LH (or forskolin), heparanase levels in CL-derived cells were not affected.

Conclusions: These findings show that heparanase is transiently up-regulated by LH during the ovulatory process and is down-regulated by the rising progesterone levels in CL, suggesting that heparanase plays a significant role in ovulation but much less so during CL development. Heparanase may be a novel member of LH-induced ECM-degrading enzymes that participate in follicular rupture.

Phospholipase D mediates hyperactivated motility in sperm capacitation

Mrs. Sarit Bar-Sheshet Itach¹ Dr. Sara Rubinstein¹ Prof. Haim Breitbart¹

¹*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University*

Introduction: Phospholipase D (PLD) catalyses the hydrolysis of phosphatidylcholine, to choline and phosphatidic acid (PA). It was recently demonstrated in our laboratory that Phospholipase D (PLD)-dependent actin polymerization is a necessary step in the cascade leading to capacitation, and it is known that capacitated sperm show hyperactivated motility. In the present study, we showed for the first correlation between PLD-dependent actin polymerization and hyperactivated motility during mouse and human sperm capacitation.

Patients / Methods: In vitro capacitation: Cauda epididymal mouse sperm were incubated in defined - capacitation medium for 90 min. Actin polymerization was measured by staining the F-actin with Phalloidin FITC, fluorescence intensity was quantified and analyzed using "Image - J" software. Sperm motility was determined by CASA using the IVOS device and manually by light microscope counting in camera sperm showing hyperactivated motility. Acrosome reaction was measured by PNA-FITC staining. IVF: metaphase II-arrested eggs and sperm were mixed and incubated for 24 hours. Fertilization rate was determined by counting the number of cleaved oocyte.

Results: Sperm incubated under capacitation conditions revealed a time dependent increase in actin polymerization followed by the development of hyperactivated motility. These two activities were blocked by the PLD inhibitor butan-1-ol but not by butan-2-ol, indicating the specificity of PLD inhibition. The activity of actin polymerization and motility in the inhibited cells, could be restored by adding PA, further indicating the involvement of PLD in these processes. Moreover, addition of exogenous PA or PLD to the cells, caused a rapid increase in actin polymerization, that was followed by a rise in the hyperactivated motility. The addition of Cytochalasin D (a known inhibitor of actin polymerization), blocked both actin polymerization and hyperactivated motility during capacitation. These results showed that actin polymerization occurs prior to the hyperactivated motility. Therefore, we speculated that the development of hyperactivated motility depends on actin polymerization. To further support this notion, we showed that phorbol ester a known Protein kinase C activator, induces actin polymerization that was followed by an increase in hyperactivated motility. Other capacitation dependent activities like AR and IVF rate are also mediated by PLD activity.

Conclusions: In conclusion, we showed that PLD-dependent actin polymerization is a critical step in the development of hyperactivated motility during mouse and human sperm capacitation.

FSH and hCG induced an increase in lipoxygenase gene expression in both human granulosa-lutein cells and cumulus cells from women undergoing in vitro fertilization

Dr. Shalom Bar-Ami^{1,2,3} Dr. Reem, Reem Biadisy-Atamny⁴ Dr. Adrian Ellenbogen⁴ Prof. Moshe Ben-Ami^{2,5} Dr. Johnny S. Younis^{2,5} Dr. Madeia, Madeia Michaeli⁴ Mrs. Orit Radin² Dr. Anat Jaffe¹

¹*Endocrinology and Diabetes Unit, Hillel-Yaffe Medical Centre, Hadera*

²*Reproductive Medicine Unit, Department of Obstetrics and Gynecology, Poriya Medical Center, Tiberias*

³*Institute of Evolution, Faculty of Science, Haifa University, Haifa*

⁴*Department of Obstetrics and Gynaecology, IVF Unit, Hillel-Yaffe Medical Centre, Hadera*

⁵*Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa*

Introduction: Two decades ago, the enzymatic products of 12 and 15 lipoxygenase were demonstrated in human granulosa lutein cells (hGLC) implicating the expression and activity of these enzymes. In later studies, inhibition of lipoxygenase by nordihydroguaiaretic acid (NDGA) reduced the LH induced progesterone (P4) secretion while addition of lipoxygenase products, such as 15HETE, overcame NDGA inhibition. In this study, we investigated the involvement of FSH and hCG in 12 lipoxygenase platelet type (12LOX), 15 type I and 15 type II lipoxygenase (15LOX-I, 15LOX-II) expression in hGLC and human cumulus cells (hCC), collected from women undergoing in vitro fertilization (IVF) treatment.

Patients / Methods: hGLC and hCC were isolated from patients undergoing IVF. hGLC were subjected to intensive cleaning from blood cells by three steps of cell sedimentation in which only clean hGLC clumps were collected followed by two successive washings by Isolate density centrifugation gradient. hCC were removed by hyaluronidase digestion and repeated pipetting and washed twice in culture medium. Cell viability was determined using 0.04% trypan blue and 100,000 and 10,000 viable hGLC and hCC, respectively, were plated in 24-well culture plates. Media was replaced on days 3 and 5 and culture was treated with FSH and hCG from day 3 of culture. Cell culture morphology was photographed and total RNA was collected on days 0, 3, 5 and 7 of culture, using the Trizol approach. Expression of 12LOX, 15LOX-I and 15LOX-II was determined by RT-PCR.

Results: Expression of 12LOX, 15LOX-I and 15LOX-II was detected by RT-PCR in both hGLC and hCC. Culture for 7 days in medium alone was associated with increased expression of the three enzymes in hGLC. In hGLC, addition of FSH, had almost no effect on 15LOX-I expression compared to control. However, FSH increased dose-dependently the expression of both 12LOX and 15LOX-II. Addition of hCG increased the expression of 15LOX-I and was less effective in increasing the expression of the other lipoxygenases. In hCC, addition of FSH increased the expression of 12LOX and 15LOX-I in culture. In all cases, both FSH and hCG induced typical changes in both hCC and hGLC cell culture morphology throughout the 7 days culture.

Conclusions: This study demonstrates the expression of 12LOX, 15LOX-I and 15LOX-II in both hCC and hGLC. The increased expression with the continuation of culture period may imply that there is a relation between the progress in cell luteinization and expression of these enzymes. In addition, the expression of these enzymes is probably under gonadotropic regulation.

מידע כללי

מקום הכנס

מרכז הכנסים כפר המכביה, רמת גן

חניה

חופשית ע"ב מקום פנוי

שפת הכנס

הכנס יתנהל בעברית; הרצאות המליאה, הסימפוזיונים בהשתתפות אורחים מחו"ל והרצאות הפרס יוצגו בשפה האנגלית, אלא אם נקבע אחרת.

תגי זיהוי ושוברי ארוחות

המשתתפים מתבקשים לענוד את תג הזיהוי במהלך הכנס, ולמסור את שובר הארוחה בעת הכניסה לאולם.

תערוכה

במקביל לכנס מתקיימות תצוגת פוסטרים ותערוכת חברות רפואיות. המשתתפים מוזמנים לבקר ולקבל מידע מהמציגים.

דמי חבר (לפקודת האגודה הישראלית לאנדוקרינולוגיה במזומן/המחאה)

◀ חבר מן המניין 120 ₪ לשנה (עד 2005 - 100 ₪ לשנה)
 ◀ גמלאי/סטודנט 40 ₪ לשנה (עד 2005 - 30 ₪ לשנה)

דמי רישום לכנס (לפקודת קשר אלדר ושות' בע"מ במזומן/המחאה/כ. אשראי)

רישום מאוחר (מ-6.4 ובמקום הכנס)			רישום ותשלום מוקדם (עד 5.4)			קבוצת רישום
17-18.4.07	18.4 בלבד	17.4 בלבד	17-18.4.07	18.4 בלבד	17.4 בלבד	יום/ימי כנס
350 ₪	200 ₪	200 ₪	250 ₪	140 ₪	140 ₪	חבר/ה מן המניין ¹
250 ₪	150 ₪	150 ₪	160 ₪	90 ₪	90 ₪	גמלאי ² וסטודנט ³
450 ₪	250 ₪	250 ₪	360 ₪	190 ₪	190 ₪	אורח ⁴ ותשלום ארגוני ⁵
<p>¹ חבר: מי שתשלום דמי חברותו התקבל בפועל עד לתום מועד הרישום המוקדם ב-5.4.07 (לרבות השלמת דמי החברות ל-3 שנים רצופות 2005-07, למעט מי שהתקבל כחבר חדש במהלך 2006-07 אשר שילם רק עבור שנה(ות) חברותו). במידה ולא יתקבל תשלום דמי החברות עד 5.4.07, ההשתתפות תחשב ע"ב אורח, בתשלום מלא. לא תבוצע הסדרת מעמד החברות במקום הכנס!</p> <p>² גמלאי: חבר אגודה, ששילם דמי חברות עד יציאתו לגמלאות, ומאז המשיך ושילם דמי חברות כגמלאי ל-3 שנים רצופות, 2005-07</p> <p>³ סטודנט: מי שלומד לתואר ראשון, שני או שלישי במדעי החיים, ומעביר יחד עם טופס התשלום עותק של תעודת סטודנט תקפה לשנת הלימודים 2006-07, במוסד אקדמי מוכר.</p> <p>⁴ אורח: כל מי שלא שילם, עד לתום מועד הרישום המוקדם (5.4.07), את דמי החברות ל-3 שנים אחרונות 2005-7 (חברים שהצטרפו לאגודה במהלך שנים אלו חייבים בדמי חבר רק משנת הצטרפותם). לא תבוצע הסדרת מעמד החברות במקום הכנס!</p> <p>⁵ תשלום ארגוני: מי שדמי הרישום משולמים בעבורו ע"י ארגון, מוסד או חברה * דמי ביטול (הביטול יעשה בכתב בלבד): עד 5.4.07 - ללא חיוב, בין 6-12.4.07 - 50₪, מ-13.4.07 חיוב מלא!</p>						הגדרות והערות

עזרה והכוונה

צוות קשר אלדר, לרשותכם בדלפקי ובאולמות הכנס, ומאחל לכולם כנס פורה ומהנה.

מזכירות הכנס

קשר אלדר ושות' בע"מ
 אירועים, כנסים ותערוכות
 ת.ד. 3636 תל אביב 61036

טל': 03-5299966, פקס: 03-5299967, דו"אל: ies@keshet.co.il