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SPECIAL FEATURE

Translational Highlights

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Abstracts

The following abstracts from The Endocrine Society journals have been selected by the editors as being particularly relevant to readers interested in translational science.

The Opposing Roles of Nitric Oxide and cGMP in the Age-Associated Decline in Rat Testicular Steroidogenesis
Srdjan J. Sokanovic, Aleksandar Z. Baburski, Marija M. Janjic, Natasa J. Stojkov, Maja M. Bjelic, Dusan Lalosevic, Silvana A. Andric, Stanko S. Stojilkovic, and Tatjana S. Kostic

The molecular mechanism of the aging-associated dysfunction of Leydig cells (LCs) is complex and poorly understood. In this study, we analyzed the contribution of nitric oxide (NO) and cGMP signaling to the age-dependent decline in LC function. Significant (>50%) decreases in serum, intratesticular, and LC androgens in aging rats (15-24 months) were accompanied by a proportional increase in NO production, an up-regulation of cGMP levels, and the expression of soluble guanylyl cyclase-1B and protein kinase G1 in LCs. In contrast, LC cAMP levels decreased with age, most likely reflecting the up-regulation of cAMP-specific phosphodiesterase expression. Moreover, the expression of genes encoding enzymes responsible for cholesterol transport and its conversion to T were reduced. Exposing LCs from aged animals to NO further increased cGMP levels and decreased cAMP and androgen production, whereas the addition of cell-permeable 8-bromoguanosine-cGMP alone had the opposite effect. In vivo inhibition of cGMP-specific phosphodiesterase-5 for 3 and 6 months in aged rats led to a partial restoration of androgens, NO, and cyclic nucleotide levels, as well as the expression of steroidogenic and NO/cGMP signaling genes. These results indicate that a progressive increase in NO production contributes to the age-dependent decrease in steroidogenesis in a cGMP-independent manner, whereas the sustained elevation in cGMP levels significantly slows the decline in LC function.

This article appears in Endocrinology, published July 24, 2013, 10.1210/en.2013-1307

Cutting Edge: Retrobulbar Inflammation, Adipogenesis, and Acute Orbital Congestion in a Preclinical Female Mouse Model of Graves' Orbitopathy Induced by Thyrotropin Receptor Plasmid-in Vivo Electroporation
Sajad Moshkelgosha, Po-Wah So, Neil Deasy, Salvador Diaz-Cano, and J Paul Banga

Graves' orbitopathy (GO) is a complication in Graves' disease (GD) but mechanistic insights into pathogenesis remain unresolved, hampered by lack of animal model. The TSH receptor (TSHR) and perhaps IGF-1 receptor (IGF-1R) are considered relevant antigens. We show that genetic immunization of human TSHR (hTSHR) A-subunit plasmid leads to extensive remodeling of orbital tissue, recapitulating GO. Female BALB/c mice immunized with hTSHR A-subunit or control plasmids by in vivo muscle electroporation were evaluated for orbital remodeling by histopathology and magnetic resonance imaging (MRI). Antibodies to TSHR and IGF-1R were present in animals challenged with hTSHR A-subunit plasmid, with predominantly TSH blocking antibodies and were profoundly hypothyroid. Orbital pathology was characterized by interstitial inflammation of extraocular muscles with CD3+ T cells, F4/80+ macrophages, and mast cells, accompanied by glycosaminoglycan deposition with resultant separation of individual muscle fibers. Some animals showed heterogeneity in orbital pathology with 1) large infiltrate surrounding the optic nerve or 2) extensive adipogenesis with expansion of retrobulbar adipose tissue. A striking finding that underpins the new model were the in vivo MRI scans of mouse orbital region that provided clear and quantifiable evidence of orbital muscle hypertrophy with protrusion (proptosis) of the eye. Additionally, eyelid manifestations of chemosis, including dilated and congested orbital blood vessels, were visually apparent. Immunization with control plasmids

failed to show any orbital pathology. Overall, these findings support TSHR as the pathogenic antigen in GO. Development of a new preclinical model will facilitate molecular investigations on GO and evaluation of new therapeutic interventions.
This article appears in Endocrinology, published July 30, 2013, 10.1210/en.2013-1576

Human Resistin in Chemotherapy-Induced Heart Failure in Humanized Male Mice and in Women Treated for Breast Cancer
Daniel R. Schwartz, Erika R. Briggs, Mohammed Qatanani, Heloisa Sawaya, Igal A. Sebag, Michael H. Picard, Marielle Scherrer-Crosbie, and Mitchell A. Lazar

Resistin is a circulating mediator of insulin resistance mainly expressed in human monocytes and responsive to inflammatory stimuli. Recent clinical studies have connected elevated resistin levels with the development and severity of heart failure. To further our understanding of the role of human resistin in heart failure, we studied a humanized mouse model lacking murine resistin but transgenic for the human Retn gene (Hum-Retn mice), which exhibits basal and inflammation-stimulated resistin levels similar to humans. Specifically, we explored whether resistin underlies acute anthracycline-induced cardiotoxicity. Remarkably, doxorubicin (25mg/kg ip) led to a 4-fold induction of serum resistin levels in Hum-Retn mice. Moreover, doxorubicin-induced cardiotoxicity was greater in the Hum-Retn mice than in littermate controls not expressing human resistin ($Retn^{-/-}$). Hum-Retn mice showed increased cardiac mRNA levels of inflammatory and cell adhesion genes compared with $Retn^{-/-}$ mice. Macrophages, but not cardiomyocytes, from Hum-Retn mice treated with doxorubicin in vitro showed dramatic induction of hRetn mRNA and protein expression. We also examined resistin levels in anthracycline-treated breast cancer patients with and without cardiotoxicity. Intriguingly, serum resistin levels in women undergoing anthracycline-containing chemotherapy increased significantly at 3 months and remained elevated at 6 months in those with subsequent cardiotoxicity. Further, elevation in resistin correlated with decline in ejection fraction in these women. These results suggest that elevated resistin is a biomarker of anthracycline-induced cardiotoxicity and may contribute in the development of heart failure via its direct effects on macrophages. These results further implicate resistin as a link between inflammation, metabolism, and heart disease.
This article appears in Endocrinology, published August 27, 2013, 10.1210/en.2013-1399

ERβ Selective Agonist Inhibits Angiotensin-Induced Cardiovascular Pathology in Female Mice
Ali Pedram, Mahnaz Razandi, Kenneth S Korach, Ramesh Narayanan, James T Dalton, and Ellis R Levin

Cardiac hypertrophy in humans can progress to cardiac failure if the underlying impetus is poorly controlled. An important direct stimulator of hypertrophy and its progression is the angiotensin II (AngII) peptide. AngII also causes hypertension that indirectly contributes to cardiac hypertrophy. Others and we have shown that estrogens acting through the estrogen receptor (ER)-β; can inhibit AngII-induced or other forms of cardiac hypertrophy in mice. However, the proliferative effects of estrogen in breast and uterus that promote the development of malignancy preclude using the steroid to prevent cardiac disease progression. We therefore tested whether an ERβ selective agonist, β-LGND2, can prevent hypertension and cardiac pathology in female mice. AngII infusion over 3 weeks significantly stimulated systolic and diastolic hypertension, cardiac hypertrophy, and cardiac fibrosis, all significantly prevented by β-LGND2 in wild-type but not in ERβ genetically deleted mice. AngII stimulated the Akt kinase to phosphorylate and inhibit the glycogen synthase kinase-3β kinase, leading to GATA4 transcription factor activation and hypertrophic mRNA expression. As a novel mechanism, all these actions were opposed by estradiol and β-LGND2. Our findings provide additional understanding of the antihypertrophic effects of ERβ and serve as an impetus to test specific receptor agonists in humans to prevent the worsening of cardiovascular disease.
This article appears in Endocrinology, published August 22, 2013, 10.1210/en.2013-1358

Testosterone Protects Against Glucotoxicity-Induced Apoptosis of Pancreatic β-Cells (INS-1) and Male Mouse Pancreatic Islets
Wanthanee Hanchang, Namoiy Semprasert, Thawornchai Limjindaporn, Pa-thai Yenchitsomanus,and Suwattanee Kooptiwut

Male hypogonadism associates with type 2 diabetes, and T can protect pancreatic β-cells from glucotoxicity. However, the protective mechanism is still unclear. This study thus aims to examine the antiapoptotic mechanism of T in pancreatic β cells cultured in high-glucose medium. T (0.0005-2 μg/mL) was added to INS-1 cells cultured in basal glucose or high-glucose media. Then cellular apoptosis, oxidative stress, and cell viability were measured. Endoplasmic reticulum (ER) stress markers and sensors and the antiapoptotic protein (B-cell lymphoma 2) were investigated by real-time PCR and Western blot analysis. ER stress markers were also measured in male mouse pancreatic islet cultured in similar conditions. T (0.05 and 0.5 μg/mL) did not have any effect on apoptosis and viability of INS-1 cells cultured in basal glucose medium, but it could reduce apoptosis and increase viability of INS-1 cells cultured in high-glucose medium. The protective effect of T is diminished by androgen receptor inhibitor. T (0.05 μg/mL) could significantly reduce nitrotyrosine levels, mRNA,

and protein levels of the ER stress markers and sensor those that were induced when INS-1 cells were cultured in high-glucose medium. It could also significantly increase the survival proteins, sarco/endoplasmic reticulum Ca^{2+} ATPase-2, and B-cell lymphoma 2 in INS-1 cells cultured in the same conditions. Similarly, it could reduce ER stress markers and increase sarco/endoplasmic reticulum Ca^{2+} ATPase protein levels in male mouse pancreatic islets cultured in high-glucose medium. T can protect against male pancreatic β-cell apoptosis from glucotoxicity via the reduction of both oxidative stress and ER stresses.

This article appears in Endocrinology, published August 22, 2013, 10.1210/en.2013-1351

Effects of the Selective Glucocorticoid Receptor Modulator Compound A on Bone Metabolism and Inflammation in Male Mice With Collagen-Induced Arthritis

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Glucocorticoids (GCs) are potent drugs to treat rheumatoid arthritis but exert adverse skeletal effects. Compound A (CpdA) is a selective GC receptor modulator with an improved risk/benefit profile in mouse models of inflammation and bone loss. Here we tested whether CpdA also exerts bone-sparing effects under proinflammatory circumstances using the collagen-induced arthritis model, a murine model of rheumatoid arthritis. CpdA decreased disease activity, paw swelling, and the paw temperature by 43%, 12%, and 7%, respectively, but was less potent than dexamethasone (DEX), which reduced these parameters by 72%, 22%, and 10%, respectively. Moreover, T cells isolated from CpdA- and DEX-treated animals were less active based on proliferation rates after challenge with type II collagen and produced smaller amounts of interferon-&ggr; and TNF as compared with T cells from PBS-treated mice. Histological assessment of the joints confirmed the weaker potency of CpdA as compared with DEX in preventing infiltration of inflammatory cells, induction of osteoclastogenesis, and destruction of articular cartilage. Due to the lack of GC-susceptible arthritis models, we were not able to fully address the bone-sparing potential of CpdA in inflammatory conditions. Nevertheless, the bone formation marker procollagen type 1 N-terminal peptide, a surrogate marker for GC-mediated suppression of bone formation, was significantly decreased by DEX in arthritic mice but not by CpdA. Our data indicate that CpdA moderately suppresses inflammation, whereas the concurrent effects on bone remain unknown. In light of its narrow therapeutic range, CpdA may be more useful as a molecular tool for dissecting GC actions rather than a therapeutic agent.

This article appears in Endocrinology, published July 24, 2013, 10.1210/en.2012-2221

Genetic Deficiency of Anti-Aging Gene *Klotho* Exacerbates Early Nephropathy in STZ-Induced Diabetes in Male Mice

Yi Lin, Makoto Kuro-o, and Zhongjie Sun

Klotho is a recently discovered anti-aging gene and is primarily expressed in kidneys. In humans, the klotho level decreases with age whereas the prevalence of chronic kidney disease (CKD) increases with age. Diabetic nephropathy is the most common form of CKD, which leads to end-stage renal disease. A decrease in klotho has been found in kidneys of patients with diabetic nephropathy. The purpose of this study is to assess whether *klotho* gene deficiency affects early diabetic nephropathy in a mouse of model of type 1 diabetes induced by streptozotocin (STZ). Male $KL^{+/-}$ mutant and wild-type mice (6-8 weeks) were injected with multiple low doses of STZ. Renal functions and renal blood flow were assessed. Kidneys were collected for histological examination and molecular assays of TGFβ1 and mammalian targets of rapamycin (mTOR) signaling. Klotho deficiency in $KL^{+/-}$ mutant mice exacerbated STZ-induced increases in urine albumin, blood urea nitrogen, expansion of mesangial matrix in renal glomeruli, and kidney hypertrophy, suggesting a protective role of klotho in kidney function and structure. Klotho deficiency did not affect renal blood flow. Notably, klotho deficiency significantly increased phosphorylation of Smad2, indicating enhanced TGFβ1 signaling in kidneys. Klotho deficiency also increased phosphorylation of mTOR and S6 (a downstream effector of mTOR), indicating enhanced mTOR signaling in kidneys of early diabetic mice. Thus, *klotho* gene deficiency may make kidneys more susceptible to diabetic injury. *Klotho* gene deficiency exacerbated early diabetic nephropathy via enhancing both TGFβ1 and mTOR signaling in kidneys.

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MK-2206, an AKT Inhibitor, Promotes Caspase-Independent Cell Death and Inhibits Leiomyoma Growth

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Uterine leiomyomas (ULs), benign tumors of the myometrium, are the number one indication for hysterectomies in the United States due to a lack of an effective alternative therapy. ULs show activation of the pro-survival AKT pathway compared with normal myometrium; however, substantial data directly linking AKT to UL cell survival are lacking. We hypothesized that AKT promotes UL cell survival and that it is a viable target for inhibiting UL growth. We used the investigational AKT inhibitor MK-2206, currently in phase II trials, on cultured primary human UL and myometrial cells, immortalized leiomyoma cells, and in leiomyoma grafts grown under the

kidney capsule in mice. MK-2206 inhibited AKT and PRAS40 phosphorylation but did not regulate serum- and glucocorticoid-induced kinase and ERK1/2, demonstrating its specificity for AKT. MK-2206 reduced UL cell viability and decreased UL tumor volumes. UL cells exhibited disruption of mitochondrial structures and underwent cell death that was independent of caspases. Additionally, mammalian target of rapamycin and p70S6K phosphorylation were reduced, indicating that mammalian target of rapamycin C1 signaling was compromised by AKT inhibition in UL cells. MK-2206 also induced autophagy in UL cells. Pretreatment of primary UL cells with 3-methyladenine enhanced MK-2206-mediated UL cell death, whereas knockdown of ATG5 and/or ATG7 did not significantly influence UL cell viability in the presence of MK-2206. Our data provide molecular evidence for the involvement of AKT in UL cell survival and suggest that AKT inhibition by MK-2206 may be a viable option to consider for the treatment of ULs.

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Targeting Stromal Androgen Receptor Suppresses Prolactin-Driven Benign Prostatic Hyperplasia (BPH)

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Stromal-epithelial interaction plays a pivotal role to mediate the normal prostate growth, the pathogenesis of benign prostatic hyperplasia (BPH), and prostate cancer development. Until now, the stromal androgen receptor (AR) functions in the BPH development, and the underlying mechanisms remain largely unknown. Here we used a genetic knockout approach to ablate stromal fibromuscular (fibroblasts and smooth muscle cells) AR in a probasin promoter-driven prolactin transgenic mouse model (Pb-PRL tg mice) that could spontaneously develop prostate hyperplasia to partially mimic human BPH development. We found Pb-PRL tg mice lacking stromal fibromuscular AR developed smaller prostates, with more marked changes in the dorsolateral prostate lobes with less proliferation index. Mechanistically, prolactin mediated hyperplastic prostate growth involved epithelial-stromal interaction through epithelial prolactin/prolactin receptor signals to regulate granulocyte macrophage-colony stimulating factor expression to facilitate stromal cell growth via sustaining signal transducer and activator of transcription-3 activity. Importantly, the stromal fibromuscular AR could modulate such epithelial-stromal interacting signals. Targeting stromal fibromuscular AR with the AR degradation enhancer, ASC-J9®, led to the reduction of prostate size, which could be used in future therapy.

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Autophagy Deficiency by Hepatic FIP200 Deletion Uncouples Steatosis From Liver Injury in NAFLD

Di Ma, Matthew M. Molusky, Jianrui Song, Chun-Rui Hu, Fang Fang, Crystal Rui, Anna V. Mathew, Subramaniam Pennathur, Fei Liu, Ji-Xin Cheng, Jun-Lin Guan, and Jiandie D. Lin

Nonalcoholic fatty liver disease is a metabolic disorder commonly associated with obesity. A subset of nonalcoholic fatty liver disease patients further develops nonalcoholic steatohepatitis that is characterized by chronic liver injury, inflammation, and fibrosis. Recent work has implicated the autophagy pathway in the mobilization and oxidation of triglycerides from lipid droplets. However, whether impaired autophagy in hepatocytes drives excess fat accumulation in the liver remains controversial. In addition, the role of autophagy in protecting the liver from gut endotoxin-induced injury has not been elucidated. Here we generated mice with liver-specific autophagy deficiency by the conditional deletion of focal adhesion kinase family kinase-interacting protein of 200 kDa (also called Rb1cc1), a core subunit of the mammalian autophagy related 1 complex. To our surprise, mice lacking FIP200 in hepatocytes were protected from starvation- and high-fat diet-induced fat accumulation in the liver and had decreased expression of genes involved in lipid metabolism. Activation of the de novo lipogenic program by liver X receptor was impaired in FIP200-deficient livers. Furthermore, liver autophagy was stimulated by exposure to low doses of lipopolysaccharides and its deficiency-sensitized mice to endotoxin-induced liver injury. Together these studies demonstrate that hepatocyte-specific autophagy deficiency per se does not exacerbate hepatic steatosis. Instead, autophagy may play a protective role in the liver after exposure to gut-derived endotoxins and its blockade may accelerate nonalcoholic steatohepatitis progression.

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CTNNB1 in Mesenchyme Regulates Epithelial Cell Differentiation during Müllerian Duct and Postnatal Uterine Development

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Müllerian duct differentiation and development into the female reproductive tract is essential for fertility, but mechanisms regulating these processes are poorly understood. WNT signaling is critical for proper development of the female reproductive tract as evident by the phenotypes of *Wnt4*, *Wnt5a*, *Wnt7a*, and β-catenin (*Ctnnb1*) mutant mice. Here we extend these findings by determining the effects of constitutive CTNNB1 activation within the mesenchyme of the

developing Müllerian duct and its differentiated derivatives. This was accomplished by crossing *Amhr2-Cre* knock-in mice with *Ctnnb1 exon (ex) 3*$^{f/f}$ mice. *Amhr2-Cre&*$^{Dgr;/+}$*; Ctnnb1 ex3*$^{f/+}$ females did not form an oviduct, had smaller uteri, endometrial gland defects, and were infertile. At the cellular level, stabilization of CTNNB1 in the mesenchyme caused alterations within the epithelium, including less proliferation, delayed uterine gland formation, and induction of an epithelial-mesenchymal transition (EMT) event. This EMT event is observed before birth and is complete within 5 days after birth. Misexpression of estrogen receptor α; in the epithelia correlated with the EMT before birth, but not after. These studies indicate that regulated CTNNB1 in mesenchyme is important for epithelial cell differentiation during female reproductive tract development.

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Uterine Deletion of *Gp130* or *Stat3* Shows Implantation Failure with Increased Estrogenic Responses

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Leukemia inhibitory factor (LIF), a downstream target of estrogen, is essential for implantation in mice. LIF function is thought to be mediated by its binding to LIF receptor (LIFR) and recruitment of coreceptor GP130 (glycoprotein 130), and this receptor complex then activates signal transducer and activator of transcription (STAT)1/3. However, the importance of LIFR and GP130 acting via STAT3 in implantation remains uncertain, because constitutive inactivation of *Lifr*, *Gp130*, or *Stat3* shows embryonic lethality in mice. To address this issue, we generated mice with conditional deletion of uterine *Gp130* or *Stat3* and show that both GP130 and STAT3 are critical for uterine receptivity and implantation. Implantation failure in these deleted mice is associated with higher uterine estrogenic responses prior to the time of implantation. These heightened estrogenic responses are not due to changes in ovarian hormone levels or expression of their nuclear receptors. In the deleted mice, estrogen-responsive gene, *Lactoferrin* (*Ltf*), and Mucin 1 protein, were up-regulated in the uterus. In addition, progesterone-responsive genes, *Hoxa10* and *Indian hedgehog* (*Ihh*), were markedly down-regulated in STAT3-inactivated uteri. These changes in uteri of deleted mice were reflected by the failure of differentiation of the luminal epithelium, which is essential for blastocyst attachment.

This article appears in Molecular Endocrinology, published July 24, 2013, 10.1210/me.2013-1086

Antiproliferative and Proapoptotic Activity of Sunitinib on Endothelial and Anaplastic Thyroid Cancer Cells via Inhibition of Akt and ERK1/2 Phosphorylation and by Down-Regulation of Cyclin-D1

Teresa Di Desidero, Anna Fioravanti, Paola Orlandi, Bastianina Canu, Riccardo Giannini, Nicla Borrelli, Shan Man, Ping Xu, Gabriella Fontanini, Fulvio Basolo, Robert S. Kerbel, Giulio Francia, Romano Danesi, and Guido Bocci

Context: Recent experimental evidence suggests a rationale for the use of multitarget tyrosine kinase inhibitors for the treatment of thyroid cancers. Sunitinib showed promising preliminary results against anaplastic thyroid cancer (ATC), and it has been used for some patients who are ineligible for clinical trials.

Objectives: The aims of this study were to investigate the in vitro and in vivo activity of sunitinib on ATC and on microvascular endothelial cells and the molecular mechanism for the observed sunitinib activity.

Methods: Proliferation and apoptotic assays were performed on human dermal microvascular endothelial and on BRAF- or H-ras-mutated ATC cells (8305C and FB3, respectively) after in vitro exposure to sunitinib for 72 hours. Vascular endothelial growth factor receptor-2, epithelial growth factor receptor, ERK1/2, and Akt phosphorylation was quantified by ELISA and Western blot. *Cyclin-D1* mRNA expression was evaluated by real-time PCR, and cyclin-D1 intracellular concentrations were measured by ELISA. 8305C tumor xenografts in nude mice were treated with sunitinib at 50 mg/kg/d (ip).

Results: Antiproliferative and proapoptotic activity of sunitinib was observed in both endothelial and ATC cells. Phospho-vascular endothelial growth factor receptor-2 levels significantly decreased after sunitinib treatment in activated endothelial cells. Phospho-epidermal growth factor receptor, ERK1/2, and Akt phosphorylation was significantly inhibited by sunitinib treatment in endothelial and cancer cells, and *cyclin-D1* mRNA and protein expression was inhibited. Sunitinib administration in vivo caused significant inhibition of tumor growth ($P < .05$).

Conclusions: Sunitinib is active in vitro and in vivo against activated endothelial and ATC cells via the inhibition of Akt and ERK1/2 phosphorylation and through the down-regulation of cyclin-D1.

This article appears in The Journal of Clinical Endocrinology & Metabolism, published August 22, 2013, 10.1210/jc.2013-1364

S100A11 Overexpression Contributes to the Malignant Phenotype of Papillary Thyroid Carcinoma

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Context: Papillary thyroid carcinoma (PTC) is the most frequent thyroid tumor and is responsible for the overall increase in thyroid cancer incidence. S100A11 (calgizzarin), a member

of the S100 Ca^{2+}-binding protein family, is involved in several different biological processes. S100A11 has been found up-regulated in PTC, both at the mRNA and protein levels.

Objective: Through a combination of expression analysis and functional in vitro and in vivo studies, we have attempted to gain insight into the relevance of *S100A11* overexpression in PTC biology.

Design: The expression of the *S100A11* gene in PTC was investigated in several gene expression data sets. The effect of *S100A11* silencing on the hallmarks of the malignant phenotype of several PTC-derived cell lines was investigated. In NIH3T3 cells, the cooperation of S100A11 with the different PTC-specific oncogenes was assessed.

Results: We found that the *S100A11* gene expression is frequently up-regulated in PTC, anaplastic thyroid carcinoma, but not in follicular thyroid carcinoma. *S100A11* overexpression was also detected in PTC-derived cell lines, which were then used for functional studies. S100A11 silencing in PTC-derived cell lines did not affect cell proliferation, whereas it reduced the loss of contact inhibition, anchorage-independent growth, and resistance to anoikis. Cotransfection experiments in NIH3T3 cells showed that overexpression of the *S100A11* gene was able to enhance the transforming capabilities of the different PTC-associated oncogenes by affecting the loss of contact inhibition, anchorage-independent growth, and in vivo tumor formation.

Conclusion: Our data indicate that S100A11 overexpression exerts a protumoral functional role in PTC pathogenesis.
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Changes in Circulating MicroRNAs Are Associated With Childhood Obesity

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Context: Circulating microRNAs (miRNAs) are valuable biomarkers of metabolic diseases and potential therapeutic targets in this field.

Objective: Our objective was to define the circulating pattern of miRNAs in childhood obesity.

Design, Settings, and Main Outcome Measure: The genome-wide circulating miRNA profile was assessed by RT-PCR in 10 boys (5 lean and 5 obese children). The most relevant miRNAs were cross-sectionally validated in 85 lean versus 40 obese children (63 boys and 62 girls) and longitudinally evaluated in samples from the same children when they were ~7 and ~10 years old (23 boys and 22 girls).

Results: The cross-sectional validation study disclosed that 15 specific circulating miRNAs were significantly deregulated in prepubertal obesity, including the decreased miR-221 and miR-28-3p and increased concentrations in plasma of miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, and miR-423-5p (all $P < .0001$). The circulating concentration of these miRNAs was significantly associated with body mass index and other measures of obesity such as percent fat mass, waist circumference, regional fat distribution and with laboratory parameters such as homeostasis model assessment of insulin resistance, high-molecular-weight adiponectin, C-reactive protein, and circulating lipids in concordance with anthropometric associations. Plasma concentrations of 10 of these circulating miRNAs changed significantly and differently during the 3-year follow-up in children who increased or decreased their normalized weight.

Conclusion: This study provides the first evidence that circulating miRNAs are deregulated in prepubertal obese children. Thus, the very early detection of an abnormal circulating miRNA profile may be a promising strategy to identify obese children who may suffer from metabolic abnormalities.
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Metreleptin Improves Blood Glucose in Patients With Insulin Receptor Mutations

Rebecca J. Brown, Elaine Cochran, and Phillip Gorden

Context: Rabson-Mendenhall syndrome (RMS) is caused by mutations of the insulin receptor and results in extreme insulin resistance and dysglycemia. Hyperglycemia in RMS is very difficult to treat, and patients are at risk for early morbidity and mortality from complications of diabetes.

Objective: Our objective was to study 1-year effects of recombinant human methionyl leptin (metreleptin) in 5 patients with RMS and 10-year effects in 2 of these patients.

Design and Setting: We conducted an open-label nonrandomized study at the National Institutes of Health.

Patients: Patients were adolescents with RMS and poorly controlled diabetes.

Intervention: Two patients were treated with escalating doses (0.02 up to 0.22 mg/kg/d) of metreleptin for 10 years, including 3 cycles of metreleptin withdrawal and reinitiation. In all 5 patients, 1-year effects of metreleptin (0.22 mg/kg/d) were studied.

Outcome Measures: Hemoglobin A1c (HbA1c) and body mass index (BMI) z-scores were evaluated every 6 months.

Results: HbA1c decreased from 11.4% ± 1.1% at baseline to 9.3% ± 1.9% after 6 months and 9.7% ± 1.6% after 12 months of metreleptin ($P = .007$). In patients treated for 10 years, HbA1c declined with each cycle of metreleptin and rose with each withdrawal. BMI z-scores declined from -1.4 ± 1.8 at baseline, to -2.6 ± 1.6 after 12 months of metreleptin

($P = .0006$). Changes in BMI z-score correlated with changes in HbA1c ($P < .0001$).

Conclusions: Metreleptin treatment for 12 months was associated with a 1.8% reduction in HbA1c; part of this improvement was likely mediated via decreased BMI. Metreleptin is a promising treatment option for RMS, but additional therapies are needed to achieve HbA1c targets.

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Interleukin-2 and Lanreotide in the Treatment of Medullary Thyroid Cancer: In Vitro and In Vivo Studies

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Context: To date no efficacious treatments are available for advanced medullary thyroid carcinoma (MTC).

Objective: We investigated in vitro and in vivo a new strategy for the therapy of MTC, combining human recombinant IL-2 with lanreotide (LAN), a somatostatin analog.

Methods: The in vitro effects of LAN on the sensitivity of TT cells, a MTC cell line, to IL-2-stimulated human peripheral blood mononuclear cells were determined by a lactate dehydrogenase release assay. In addition, we evaluated the toxicity, the effects on quality of life, and the antitumor activity of sc low-dose IL-2 in combination with LAN (90 mg every 28 days) in a series of 6 patients with symptomatic and advanced MTC.

Results: The cytotoxicity of IL-2-activated peripheral blood mononuclear cells was significantly increased in TT cells treated with LAN or LAN plus IL-2 compared with that in TT cells without treatment. The therapy was well tolerated, and a statistically significant improvement of quality of life was observed in patients treated with the combination of LAN and IL-2. After 6 months of therapy, partial response and stable disease have been recorded in 2 and 3 patients, respectively, with a significant decrease in calcitonin levels in 3 patients.

Conclusions: Both in vitro and in vivo evidence suggests that the combination of LAN and IL-2 may have a role in the management of advanced and symptomatic MTC. However, these preliminary data require further validation in larger randomized trials.

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Does Inflammation Determine Metabolic Health Status in Obese and Nonobese Adults?

Catherine M. Phillips and Ivan J. Perry

Context: Inflammation is a potential mechanism linking obesity and cardiometabolic risk. Limited data on inflammatory markers in metabolically healthy obese and nonobese individuals exist.

Objective: The aim of the study was to investigate the extent to which differences between metabolically healthy and unhealthy obese and nonobese adults, defined using a range of metabolic health definitions, are correlated with a range of inflammatory markers.

Design: A cross-sectional sample of 2047 men and women aged 45-74 years participated in the study. Participants were classified as obese (body mass index ≥ 30 kg/m^2) and nonobese (body mass index < 30 kg/m^2). Metabolic health status was defined using 5 existing metabolic health definitions based on a range of cardiometabolic abnormalities. Serum acute-phase reactants, adipocytokines, proinflammatory cytokines, and white blood cell counts were determined.

Results: According to most definitions, metabolically healthy obese and nonobese individuals presented with lower concentrations of complement component 3, C-reactive protein, TNF-α;, IL-6, and plasminogen activator inhibitor-1; higher adiponectin levels; and reduced white blood cell count compared to their metabolically unhealthy counterparts. Logistic regression analysis identified greater likelihood of metabolically healthy obesity among individuals with lower levels of complement component 3 (odds ratios [ORs], 2-3.5), IL-6 (ORs, 1.7-2.9), plasminogen activator inhibitor-1 (ORs, 1.7-2.9), and white blood cells (ORs, 2.1-2.5) and higher adiponectin concentrations (ORs, 2.6-4.0).

Conclusions: Favorable inflammatory status is positively associated with metabolic health in obese and nonobese individuals. These findings are of public health and clinical significance in terms of screening and stratification based on metabolic health phenotype to identify those at greatest cardiometabolic risk for whom appropriate therapeutic or intervention strategies should be developed.

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PTH: A New Target in Arteriosclerosis?

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Context: Growing evidence demonstrates that hyperparathyroidism is associated with an increased risk of cardiovascular morbidity and mortality. However, little is known about the relation between serum PTH levels within the normal range and cardiovascular diseases (CVDs).

Objective: In this study the relationship of serum PTH levels within the normal range with CVD and abdominal aortic calcifications was investigated.

Design: A cross-sectional, population-based study was performed using data of the Longitudinal Aging Study Amsterdam, including 558 men and 537 women, aged 65-88 years. Models were controlled for sex, age, body mass index, hypertension, diabetes mellitus, high-density lipoprotein cholesterol, total cholesterol, smoking, physical activity, alcohol consumption, glomerular filtration rate, season of blood collection, calcium or diuretic use, and serum 25-hydroxyvitamin D and osteocalcin levels when these variables were found to be relevant confounders.

Results: Multivariate models showed that subjects in the highest quintile of serum PTH had a significantly higher risk of CVD as compared with subjects in the lowest quintile (odds ratio 2.22, confidence interval 1.39-3.56). The relationship between PTH and abdominal aortic calcifications was observed only in men, which remained significant after adjusting for confounding (odds ratio 4.03, confidence interval 1.50-10.83).

Conclusions: This study demonstrated that in older persons the presence of serum PTH levels within the upper normal range is highly related to CVD. In men, this association may partly be explained by calcifications of the abdominal aorta. Because CVD poses an important health risk, further elucidation of the role of serum PTH in CVD and arteriosclerosis is relevant.

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Parallel Variations of Insulin-Like Peptide 3 (INSL3) and Antimüllerian Hormone (AMH) in Women With the Polycystic Ovary Syndrome According to Menstrual Cycle Pattern

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Context: Antimüllerian hormone (AMH) and insulin-like factor 3 (INSL3) represent ovarian functional markers of granulosa and theca cells, respectively.

Objective: We conducted a prospective study to investigate AMH and INSL3 plasma levels in 3 groups of women with polycystic ovary syndrome (PCOS) classified according to menstrual cyclicity pattern and their relationship with ovarian morphology and hormonal levels.

Design and Participants: AMH and INSL3 were measured in a cohort of 57 patients with PCOS, divided into 3 groups according to menstrual status: eumenorrheic (PCOS-E, n = 15), oligomenorrheic (PCOS-O, n = 25), and amenorrheic (PCOS-A, n = 17). Clinical and endocrine characteristics and ovarian morphology were compared among the groups. Twenty-seven age- and weight-matched women without hyperandrogenism were included as controls.

Results: According to the menstrual pattern, the women with PCOS-A and PCOS-O had higher INSL3 levels with respect to the control women ($P = .025$ and $P = .004$, respectively) and higher but not significant INSL3 levels compared with those of the women with PCOS-E. AMH levels were significantly higher in women with PCOS-A and PCOS-O with respect to those in women with PCOS-E ($P < .001$ and $P < .001$, respectively) and control women ($P < .001$ and $P < .001$, respectively). Interestingly, a significant positive correlation was found between INSL3 and AMH blood levels in all women with PCOS ($R = 0.43$; $P = .002$) and across the groups ($R = 0.41$; $P < .001$).

Conclusions: INSL3 and AMH levels are significantly correlated with each other in women with PCOS, and they are significantly increased, particularly in the presence of amenorrhea and oligomenorrhea. INSL3 and AMH may reflect a dysfunction of PCOS thecal and granulosa cells, which are responsible for the increased androgen production and chronic anovulation of this condition.

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Skeletal Muscle MnSOD, Mitochondrial Complex II, and SIRT3 Enzyme Activities Are Decreased in Maternal Obesity During Human Pregnancy and Gestational Diabetes Mellitus

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Context: Insulin resistance and systemic oxidative stress are prominent features of pregnancies complicated by maternal obesity or gestational diabetes mellitus (GDM). The role of skeletal muscle oxidative stress or mitochondrial capacity in obese pregnant women or obese women with GDM is unknown.

Objective: We investigated whether obese pregnant women, compared with normal weight (NW) pregnant women, demonstrate decreased skeletal muscle mitochondrial enzyme activity and elevated markers of oxidative stress, and if these differences are more severe in obese women diagnosed with GDM.

Design: We measured mitochondrial enzyme activity and markers of oxidative stress in skeletal muscle tissue from NW pregnant women (n = 10), obese pregnant women with normal glucose tolerance (NGT; n = 10), and obese pregnant women with GDM (n = 8), undergoing cesarean delivery (~37 wk gestation).

Results: Electron transport complex-II and manganese superoxide dismutase (MnSOD) enzyme activities were decreased in obese-NGT and obese-GDM, compared with NW women. The glutathione redox ratio (GSH:GSSG) was decreased in obese-NGT and obese-GDM, indicative of

increased oxidative stress. Mitochondrial sirtuin (SIRT)3 mRNA content and enzyme activity were lower in skeletal muscle of obese-NGT and obese-GDM women. Importantly, acetylation of MnSOD, a SIRT3 target, was increased in obese-NGT and obese-GDM vs NW women and was inversely correlated with SIRT3 activity (r = -0.603), suggesting a mechanism for reduced MnSOD activity.

Conclusions: These data show that obese pregnant women demonstrate decreased skeletal muscle mitochondrial respiratory chain enzyme activity and decreased mitochondrial antioxidant defense. Furthermore, reduced skeletal muscle SIRT3 activity may play a role in the increased oxidative stress associated with pregnancies complicated by obesity.

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Paternal *GNAS* Mutations Lead to Severe Intrauterine Growth Retardation (IUGR) and Provide Evidence for a Role of XLαs in Fetal Development

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Context: Heterozygous *GNAS* inactivating mutations cause pseudohypoparathyroidism type Ia (PHP-Ia) when maternally inherited and pseudopseudohypoparathyroidism (PPHP)/progressive osseous heteroplasia (POH) when paternally inherited. Recent studies have suggested that mutations on the paternal, but not the maternal, *GNAS* allele could be associated with intrauterine growth retardation (IUGR) and thus small size for gestational age.

Objectives: The aim of the study was to confirm and expand these findings in a large number of patients presenting with either PHP-Ia or PPHP/POH.

Patients and Methods: We collected birth parameters (ie, gestational age, weight, length, and head circumference) of patients with either PHP-Ia (n = 29) or PPHP/POH (n = 26) with verified *GNAS* mutations. The parental allele carrying the mutation was assessed by investigating the parents or, when a de novo mutation was identified, through informative intragenic polymorphisms.

Results: Heterozygous *GNAS* mutations on either parental allele were associated with IUGR. However, when these mutations are located on the paternal *GNAS* allele, IUGR was considerably more pronounced than with mutations on the maternal allele. Moreover, birth weights were lower with paternal *GNAS* mutations affecting exons 2-13 than with exon 1/intron 1 mutations.

Conclusions: These data indicate that a paternally derived *GNAS* transcript, possibly XLαs, is required for normal fetal growth and development and that this transcript affects placental functions. Thus, similar to other imprinted genes, *GNAS* controls growth and/or fetal development.

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