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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.

Diacerhein Improves Glucose Tolerance and Insulin Sensitivity in Mice on a High-Fat Diet

Natália Tobar, Alexandre G. Oliveira, Dioze Guadagnini, Renata A. Bagarolli, Guilherme Z. Rocha, Tiago G. Araújo, Junia C. R. Martins, Ricardo L. Zollner, Luiz H. B. Boechat, José B. C. Carvalheira, Patrícia O. Prada, and Mario J. A. Saad

Obesity and type 2 diabetes are characterized by insulin resistance, and the common basis of these events is a chronic and systemic inflammatory process marked by the activation of the c-Jun N-terminal kinase (JNK) and inhibitor-κB kinase (IKKβ)/nuclear factor-κB (NFκB) pathways, up-regulated cytokine synthesis, and endoplasmic reticulum dysfunction. The aim of this study was to evaluate the effects of diacerhein administration, an antiinflammatory drug that reduces the levels of inflammatory cytokines, on insulin sensitivity and signaling in diet-induced obese (DIO) mice. Swiss mice were fed with conventional chow (control group) or a high-fat diet (DIO group). Later, DIO mice were randomly subdivided into a new subgroup (DAR) that received 20 mg/kg diacerhein for 10 day. Western blotting was used to quantify the expression and phosphorylation of insulin receptor, insulin receptor substrate 1, and Akt and of inflammatory mediators that modulate insulin signaling in a negative manner (IKKβ, JNK, and inducible nitric oxide synthase). We show here, for the first time, that the administration of diacerhein in DIO mice improved endoplasmic reticulum stress, reduced JNK and IKKβ phosphorylation, and resulted in a marked improvement in fasting glucose, a decrease in macrophage infiltration in adipose tissue, and a reduced expression and activity of proinflammatory mediators accompanied by an improvement in the insulin signaling mainly in the liver and adipose tissue. Taken together, these results indicate that diacerhein treatment improves insulin sensitivity in obesity, mediated by the reversal of subclinical inflammation, and that this drug may be an alternative therapy for insulin resistance.

This article appears in Endocrinology, published September 6, 2011, 10.1210/en.2011-0249

The Phytoestrogen Genistein Is a Tissue-Specific Androgen Receptor Modulator

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To enable studies of androgen signaling in different tissues in vivo, we generated an androgen receptor (AR) reporter mouse line by inserting a *luciferase* gene construct into the murine genome. The construct is driven by four copies of androgen-responsive elements from the mouse *sex-limited protein* gene (*slp*-HRE2) and a minimal *thymidine kinase* promoter. Luciferase activity was readily measurable in a number of murine tissues, including prostate, lung, testis, brain, and skeletal muscle, and testosterone administration elicited a significant increase in reporter gene activity in these tissues. Consumption of isoflavonoid genistein is linked to reduced risk of prostate cancer, but direct effects of genistein on the AR pathway are not well understood. To examine androgen-modulating activity of genistein in vivo, male mice received daily doses of genistein (10 mg/kg) for 5 day. In intact males, genistein was antiandrogenic in testis, prostate, and brain, and it attenuated reporter gene activity by 50–80%. In castrated males, genistein exhibited significant androgen agonistic activity in prostate and brain by increasing reporter gene activity over 2-fold in both tissues. No antiandrogenic action was seen in lung or skeletal muscle of intact males. Gene expression profiling of the murine prostate under the same experimental conditions revealed

that genistein modulates androgen-dependent transcription program in prostate in a fashion similar to that observed in reporter mice by *luciferase* expression. In conclusion, genistein is a partial androgen agonist/antagonist in some but not in all mouse tissues and should be considered as a tissue-specific AR modulator.

This article appears in Endocrinology, published August 30, 2011, 10.1210/en.2011-0221

From Pituitary Expansion to Empty Sella: Disease Progression in a Mouse Model of Autoimmune Hypophysitis

Isabella Lupi, Jiangyang Zhang, Angelika Gutenberg, Melissa Landek-Salgado, Shey-Cherng Tzou, Susumu Mori, and Patrizio Caturegli

Lymphocytic hypophysitis has a variable clinical course, where a swelling of the pituitary gland at presentation is thought to be followed by pituitary atrophy and empty sella. Data in patients, however, are scanty and contradictory. To better define the course of hypophysitis, we used an experimental model based on the injection of pituitary proteins into SJL mice. A cohort of 33 mice was divided into three groups: 18 cases were immunized with pituitary proteins emulsified in complete Freund's adjuvant; six controls were injected with adjuvant only; and nine controls were left untreated. Mice were followed by cranial magnetic resonance imaging (MRI) for up to 300 day, for a total of 106 MRI scans, and killed at different time points to correlate radiological and pathological findings. Empty sella was defined as a reduction in pituitary volume greater than 2 SD below the mean volume. All immunized mice showed by MRI a significant expansion of pituitary volume during the early phases of the disease. The volume then decreased gradually in the majority of cases (14 of 18, 78%), reaching empty sella values by d 300 after immunization. In a minority of cases (four of 18, 22%), the decrease was so rapid and marked to induce a central area of necrosis accompanied by hemorrhages, mimicking the condition known in patients as pituitary apoplexy. No radiological or pathological changes were observed in controls. Overall, these findings indicate that the evolution of hypophysitis is complex but can lead, through different routes, to the development of empty sella.

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A Rapid Release of Corticosteroid-Binding Globulin from the Liver Restrains the Glucocorticoid Hormone Response to Acute Stress

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A strict control of glucocorticoid hormone responses to stress is essential for health. In blood, glucocorticoid hormones are for the largest part bound to corticosteroid-binding globulin (CBG), and just a minor fraction of hormone is free. Only free glucocorticoid hormone is able to exert biological effects, but little is known about its regulation during stress. We found, using a dual-probe in vivo microdialysis method, that in rats, the forced-swim stress-induced rise in free corticosterone (its major glucocorticoid hormone) is strikingly similar in the blood and in target compartments such as the sc tissue and the brain. However, in all compartments, the free corticosterone response was delayed by 20–30 min as compared with the total corticosterone response in the blood. We discovered that CBG is the key player in this delay. Swim stress evoked a fast (within 5 min) and profound rise in CBG protein and binding capacity in the blood through a release of the protein from the liver. Thus, the increase in circulating CBG levels after stress restrains the rise in free corticosterone concentrations for approximately 20 min in the face of mounting total hormone levels in the circulation. The stress-induced increase in CBG seems to be specific for moderate and strong stressors. Both restraint stress and forced swimming caused an increase in circulating CBG, whereas its levels were not affected by mild novelty stress. Our data uncover a new, highly dynamic role for CBG in the regulation of glucocorticoid hormone physiology after acute stress.

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Study of the Potential Association of Adipose Tissue GLP-1 Receptor with Obesity and Insulin Resistance

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The increase in glucagon-like peptide-1 (GLP-1) activity has emerged as a useful therapeutic tool for the treatment of type 2 diabetes mellitus. The actions of GLP-1 on β-cells and the nervous and digestive systems are well known. The action of this peptide in adipose tissue (AT), however, is still poorly defined. Furthermore, no relationship has been established between GLP-1 receptor (GLP-1R) in AT and obesity and insulin resistance (IR). We provide evidence for the presence of this receptor in AT and show that its mRNA and protein expressions are increased in visceral adipose depots from morbidly obese patients with a high degree of IR. Experiments with the 3 T3-L1 cell line showed the lipolytic and lipogenic dose-dependent effect of GLP-1. Moreover, GLP-1 stimulated lipolysis in 3 T3-L1 adipocytes in a receptor-dependent manner involving downstream adenylate cyclase/cAMP signaling. Our data also demonstrate that the expression of the GLP-1R in AT correlated positively with the homeostasis model assessment index in obese IR subjects. Furthermore, prospective studies carried out with

patients that underwent biliopancreatic diversion surgery showed that subjects with high levels of GLP-1R expression in AT, which indicates a deficit of GLP-1 in this tissue, were those whose insulin sensitivity improved after surgery, suggesting the potential relationship between AT GLP-1R and insulin sensitivity amelioration in obese subjects. Altogether these results indicate that the GLP-1/GLP-1R system in AT represents another potential candidate for improving insulin sensitivity in obese patients.

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Exposure to Chronic Pregnancy Stress Reverses Peripartum-Associated Adaptations: Implications for Postpartum Anxiety and Mood Disorders

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Maternal adaptations, such as decreased anxiety and attenuated stress responsiveness, are necessary to enable successful postnatal development of the offspring. However, there is growing evidence that they are also required to protect the mental health of the mother and that exposure to chronic stress during pregnancy may prevent such adaptations. Overcrowding stress (24 h) and restraint stress (2×1 h) were employed on alternate days between pregnancy d 4–16 to examine the impact of chronic pregnancy stress on relevant behavioral, neuroendocrine, and neuronal peripartum adaptations. To determine whether the chronic stress-induced alterations were specific to the peripartum period, we included virgins as controls. Validating the stress procedure, we demonstrated decreased body-weight gain and increased adrenal weight in stressed dams, relative to their unstressed controls. Chronic stress prevented a number of peripartum adaptations, including basal plasma hypercorticosterone levels, increased oxytocin mRNA expression in the hypothalamic para-ventricular nucleus, and anxiolysis. However, chronic stress did not prevent the peripartum-associated decrease in CRH mRNA expression or attenuate corticosterone response to an acute stressor, nor did it affect hypothalamic vasopressin mRNA expression. Illustrating the specificity of these stress-induced changes to the peripartum period, none of these parameters were affected in stressed virgins. Although chronic stress did not alter depression-related behavior, it reversed the response to acute imipramine treatment and increased active maternal behavior in lactation. Thus, prevention of the peripartum-associated increases in basal corticosterone and oxytocin system activity by pregnancy stress reveal two alterations that may increase the risk of postpartum psychiatric disorders, specifically anxiety.

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Maternal Diabetes Compromises the Organization of Hypothalamic Feeding Circuits and Impairs Leptin Sensitivity in Offspring

Sophie M. Steculorum and Sebastien G. Bouret

Maternal diabetes is a common complication of pregnancy, and the offspring of diabetic mothers have a higher risk of developing obesity and type 2 diabetes later in life. Despite these observations, the precise biological processes mediating this metabolic programming are not well understood. Here, we explored the consequences of maternal diabetes on the organization of hypothalamic neural circuits involved in the regulation of energy balance. To accomplish this aim, we used a mouse model of maternal insulin deficiency induced by streptozotocin injections. Maternal diabetes was found to be associated with changes in offspring growth as revealed by a significantly higher pre-and postweaning body weight in the offspring of insulin-deficient dams relative to those of control mice. Mice born to diabetic dams also showed increased fasting glucose levels, increased insulin levels, and increased food intake during their adult lives. These impairments in metabolic regulation were associated with leptin resistance during adulthood. Importantly, the ability of leptin to activate intracellular signaling in arcuate neurons was also significantly reduced in neonates born to diabetic dams. Furthermore, neural projections from the arcuate nucleus to the paraventricular nucleus were markedly reduced in the offspring of insulin-deficient dams. Together, these data show that insulin deficiency during gestation has long-term consequences for metabolic regulation. They also indicate that animals born to diabetic dams display abnormally organized hypothalamic feeding pathways that could result from the attenuated responsiveness of hypothalamic neurons to the neurotrophic actions of leptin during neonatal development.

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Developmental and Cell-Specific Expression of Thyroid Hormone Transporters in the Mouse Cochlea

David S. Sharlin, Theo J. Visser, and Douglas Forrest

Thyroid hormone is essential for the development of the cochlea and auditory function. Cochlear response tissues, which express thyroid hormone receptor β (encoded by *Thrb*), include the greater epithelial ridge and sensory epithelium residing inside the bony labyrinth. However, these response tissues lack direct blood flow, implying that mechanisms exist to shuttle hormone from the circulation to target tissues. Therefore, we investigated expression of candidate thyroid hormone transporters L-type amino acid transporter 1, monocarboxylate transporter (Mct)8, Mct10, and organic anion transporting polypeptide 1c1 in mouse cochlear development by in situ hybridization and immunofluorescence analysis. L-type amino acid transporter 1 localized to cochlear blood vessels and transiently to sensory hair cells. Mct8

localized to the greater epithelial ridge, tympanic border cells underlying the sensory epithelium, spiral ligament fibrocytes, and spiral ganglion neurons, partly overlapping with the *Thrb* expression pattern. Mct10 was detected in a highly restricted pattern in the outer sulcus epithelium and weakly in tympanic border cells and hair cells. Organic anion transporting polypeptide 1c1 localized primarily to fibrocytes in vascularized tissues of the spiral limbus and spiral ligament and to tympanic border cells. Investigation of hypothyroid *Tshr*$^{-/-}$mice showed that transporter expression was delayed consistent with retardation of cochlear tissue maturation but not with compensatory responses to hypothyroidism. The results demonstrate specific expression of thyroid hormone transporters in the cochlea and suggest that a network of thyroid hormone transport underlies cochlear development.

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Maternal Obesity Promotes a Proinflammatory Signature in Rat Uterus and Blastocyst

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Maternal obesity at conception increases the risk of offspring obesity, thus propagating an intergenerational vicious cycle. Male offspring born to obese dams are hyperresponsive to high fat-diets, gaining greater body weight, fat mass, and additional metabolic sequelae. In this report, we identify the impact of maternal obesity before conception, on the embryo, and intrauterine milieu during the periimplantation period. We conducted global transcriptomic profiling in the uterus and periimplantation blastocyst, gene/protein expression analyses of inflammatory pathways in conjunction with endocrine and metabolic characterization in the dams at implantation. Uterine gene expression profiles of lean and obese dams revealed distinct signatures for genes regulating inflammation and lipid metabolism. Both pathway and gene-set enrichment analysis revealed uterine nuclear factor-κB and c-Jun N-terminal kinase signaling to be up-regulated in the uterus of obese dams, which was confirmed via immunoblotting. Obese uteri also evidenced an inflammatory secretome with higher chemokine mRNA abundance (CCL2, CCL5, CCL7, and CxCL10) and related regulators (TLR2, CD14, and Ccr1). Increased inflammation in the uterus was associated with ectopic lipid accumulation and expression of lipid metabolic genes. Gene expression in sex-identified male periimplantation blastocyst at day postcoitum 4.5 was clearly influenced by maternal obesity (359 transcripts, ± 1.4-fold), including changes in developmental and epigenetic regulators. Akin to the uterus, nuclear factor-κB-regulated pro-inflammatory genes (CCL4 and CCL5) increased and expression of antioxidant (GPx3) and mitochondrial (TFAM and NRF1) genes decreased in the obese embryos. Our results suggest that ectopic lipid and inflammation may link maternal obesity to increased predisposition of offspring to obesity later in life.

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GH, But Not GHRH, Plays a Role in the Development of Experimental Autoimmune Encephalomyelitis

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GH has been suggested to influence the function of the immune system in several species. Experimental autoimmune encephalomyelitis (EAE) (an animal model for multiple sclerosis) has been reported not to occur in GH-deficient (GHD) mice. The aim of this study was to elucidate the effects of GH and GHRH replacement on development of EAE in a mouse model of isolated GHD due to removal of the GHRH gene [GHRH knockout (GHRHKO)]. We studied two groups of adult female mice: 12 GH-sufficient animals (control) and 36 GHRHKO animals. All mice were immunized with myelin oligodendrocyte glycoprotein peptide, a peptide known to induce EAE. GHRHKO mice were left untreated or were treated for 4 week with daily sc injections of recombinant GH or of a GHRH super agonist JI-38 (JI38-GHD). Evaluation of EAE symptoms was carried out daily, and T-proliferative assay and histopathological analysis of the spinal cord were performed. GHRHKO mice were less prone to develop EAE when compared with control mice. GH (but not JI-38) restored the original susceptibility of mice to the disease, despite lack of complete serum IGF-I normalization. GH treatment was also associated with a markedly increase in spleen size and T-cell proliferation specific to myelin oligodendrocyte glycoprotein peptide. GH (but not GHRH) plays an important role in the development of EAE.

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The Estrogen-Regulated Transcription Factor *PITX1* Coordinates Gene-Specific Regulation by Estrogen Receptor-α in Breast Cancer Cells

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The estrogen receptor α (ERα) is a master regulator of gene expression and works along with cooperating transcription factors in mediating the actions of the hormone estradiol (E2) in ER-positive tissues and breast tumors. Here, we report that expression of paired-like homeodomain transcription factor (*PITX1*), a tumor suppressor and member of the homeobox family of transcription factors, is robustly up-regulated by E2 in several ERα-positive breast cancer cell lines via ERα-dependent interaction between the proximal promoter and an

enhancer region 5′ upstream of the *PITX1* gene. Overexpression of *PITX1* selectively inhibited the transcriptional activity of ERα and ERβ, while enhancing the activities of the glucocorticoid receptor and progesterone receptor. Reduction of *PITX1* by small interfering RNA enhanced ERα-dependent transcriptional regulation of a subset of ERα target genes. The consensus *PITX1* binding motif was found to be present in 28% of genome-wide ERα binding sites and was in close proximity to estrogen response elements in a subset of ERα binding sites, and E2 treatment enhanced *PITX1* as well as ERα recruitment to these binding sites. These studies identify *PITX1* as a new ERα transcriptional target that acts as a repressor to coordinate and fine tune target-specific, ERα-mediated transcriptional activity in human breast cancer cells.

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Differential Role of PKA Catalytic Subunits in Mediating Phenotypes Caused by Knockout of the Carney Complex Gene *Prkar1a*

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The Carney complex is an inherited tumor predisposition caused by activation of the cAMP-dependent protein kinase [protein kinase A (PKA)] resulting from mutation of the PKA-regulatory subunit gene *PRKAR1A*. Myxomas and tumors in cAMP-responsive tissues are cardinal features of this syndrome, which is unsurprising given the important role played by PKA in modulating cell growth and function. Previous studies demonstrated that cardiac-specific knockout of *Prkar1a* causes embryonic heart failure and myxomatous degeneration in the heart, whereas limited Schwann cell-specific knockout of the gene causes schwannoma formation. In this study, we sought to determine the role of PKA activation in this phenotype by using genetic means to reduce PKA enzymatic activity. To accomplish this goal, we introduced null alleles of the PKA catalytic *Prkaca* (Ca) or *Prkacb* (Cb) into the Prkar1a-cardiac knockout (R1a-CKO) or limited Schwann cell knockout (R1a-TEC3KO) line. Heterozygosity for *Prkaca* rescued the embryonic lethality of the R1a-CKO, although mice had a shorter than normal lifespan and died from cardiac failure with atrial thrombosis. In contrast, heterozygosity for *Prkacb* only enabled the mice to survive 1 extra day during embryogenesis. Biochemical analysis indicated that reduction of Ca markedly reduced PKA activity in embryonic hearts, whereas reduction of Cb had minimal effects. In R1a-TEC3KO mice, tumorigenesis was completely suppressed by a heterozygosity for *Prkaca*, and by more than 80% by heterozygosity for Prkarcb. These data suggest that both developmental and tumor phenotypes caused by *Prkar1a* mutation result from excess PKA activity due to PKA-Ca.

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Connective Tissue Growth Factor Is Required for Normal Follicle Development and Ovulation

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Connective tissue growth factor (CTGF) is a cysteine-rich protein the synthesis and secretion of which are hypothesized to be selectively regulated by activins and other members of the TGF-β superfamily. To investigate the in vivo roles of CTGF in female reproduction, we generated *Ctgf* ovarian and uterine conditional knockout (cKO) mice. *Ctgf* cKO mice exhibit severe subfertility and multiple reproductive defects including disrupted follicle development, decreased ovulation rates, increased numbers of corpus luteum, and smaller but functionally normal uterine horns. Steroidogenesis is disrupted in the *Ctgf* cKO mice, leading to increased levels of serum progesterone. We show that disrupted follicle development is accompanied by a significant increase in granulosa cell apoptosis. Moreover, despite normal cumulus expansion, *Ctgf* cKO mice exhibit a significant decrease in oocytes ovulated, likely due to impaired ovulatory process. During analyses of mRNA expression, we discovered that *Ctgf* cKO granulosa cells show gene expression changes similar to our previously reported granulosa cell-specific knockouts of *activin* and *Smad4*, the common TGF-β family intracellular signaling protein. We also discovered a significant down-regulation of *Adamts1*, a progesterone-regulated gene that is critical for the remodeling of extracellular matrix surrounding granulosa cells of preovulatory follicles. These findings demonstrate that CTGF is a downstream mediator in TGF-β and progesterone signaling cascades and is necessary for normal follicle development and ovulation.

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cMyc Is a Principal Upstream Driver of β-Cell Proliferation in Rat Insulinoma Cell Lines and Is an Effective Mediator of Human β-Cell Replication

Esra Karslioglu, Jeffrey W. Kleinberger, Fatimah G. Salim, Amy E. Cox, Karen K. Takane, Donald K. Scott, and Andrew F. Stewart

Adult human β-cells replicate slowly. Also, despite the abundance of rodent β-cell lines, there are no human β-cell lines for diabetes research or therapy. Prior studies in four commonly studied rodent β-cell lines revealed that all four lines displayed an unusual, but strongly reproducible, cell cycle signature: an increase in seven G_1/S molecules, i.e. cyclins A, D3, and E, and cdk1, -2, -4, and −6. Here, we explore the upstream mechanism(s) that drive these cell

cycle changes. Exploration of candidate upstream molecules in Ins1 and RIN cells; biochemical, pharmacological and molecular delineation of the role of cMyc in driving rodent cell cycle molecules and proliferation; human β-cell proliferation and survival. Our results indicate that cMyc is: 1) uniquely up-regulated among other candidates; 2) principally responsible for the increase in the seven G_1/S molecules; and, 3) largely responsible for proliferation in rat β-cell lines. Importantly, cMyc expression in β-cell lines, although some 5-to 7-fold higher than normal rat β-cells, is far below the levels (75-to 150-fold) previously associated with β-cell death and dedifferentiation. Notably, modest overexpression of cMyc is able to drive proliferation without cell death in normal rat and human β-cells. We conclude that cMyc is an important driver of replication in the two most commonly employed rat β-cell lines. These studies reverse the current paradigm in which cMyc overexpression is inevitably associated with β-cell death and dedifferentiation. The cMyc pathway provides potential approaches, targets, and tools for driving and sustaining human β-cell replication.

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In Silico Analysis Identifies a Novel Role for Androgens in the Regulation of Human Endometrial Apoptosis

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Context: The endometrium is a multicellular, steroid-responsive tissue that undergoes dynamic remodeling every menstrual cycle in preparation for implantation and, in absence of pregnancy, menstruation. Androgen receptors are present in the endometrium.

Objective: The objective of the study was to investigate the impact of androgens on human endometrial stromal cells (hESC).

Design: Bioinformatics was used to identify an androgen-regulated gene set and processes associated with their function. Regulation of target genes and impact of androgens on cell function were validated using primary hESC.

Setting: The study was conducted at the University Research Institute.

Patients: Endometrium was collected from women with regular menses; tissues were used for recovery of cells, total mRNA, or protein and for immunohistochemistry.

Results: A new endometrial androgen target gene set (n=15) was identified. Bioinformatics revealed 12 of these genes interacted in one pathway and identified an association with control of cell survival. Dynamic androgen-dependent changes in expression of the gene set were detected in hESC with nine significantly down-regulated at 2 and/or 8 h. Treatment of hESC with dihydrotestosterone reduced staurosporine-induced apoptosis and cell migration/proliferation.

Conclusions: Rigorous in silico analysis resulted in identification of a group of androgen-regulated genes expressed in human endometrium. Pathway analysis and functional assays suggest androgen-dependent changes in gene expression may have a significant impact on stromal cell proliferation, migration, and survival. These data provide the platform for further studies on the role of circulatory or local androgens in the regulation of endometrial function and identify androgens as candidates in the pathogenesis of common endometrial disorders including polycystic ovarian syndrome, cancer, and endometriosis.

This article appears in The Journal of Clinical Endocrinology & Metabolism, published August 24, 2011, 10.1210/jc.2011-0272

Early Initiation of Hormone Therapy in Menopausal Women Is Associated with Increased Hippocampal and Posterior Cingulate Cholinergic Activity

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Context: The role of ovarian hormones in maintaining neuronal integrity and cognitive function is still debated. This study was undertaken to clarify the potential relationship between postmenopausal hormone use and the cholinergic system.

Objective: We hypothesized that early initiated hormone therapy (HT) preserves the cholinergic system and that estrogen therapy (ET) would be associated with higher levels of acetylcholinesterase activity in the posterior cingulate cortex and hippocampus compared to estrogen plus progestin therapy (EPT) or no HT.

Design and Setting: We conducted a cross-sectional study at a university teaching hospital.

Patients: Fifty postmenopausal women (age, 65.2± 0.7 year) with early long-term HT (n=34; 13 ET and 21 EPT) or no HT (n=16) participated in the study.

Interventions: There were no interventions.

Main Outcome Measure: We measured cholinergic activity (acetylcholinesterase) in the hippocampus and posterior cingulate brain regions as measured by N-[C] methylpiperidin-4-yl propionate and positron emission tomography as a marker of cholinergic function.

Results: Significant effects of treatment on cholinergic activity measures were obtained in the left hippocampus (F=3.56; P=0.04), right hippocampus (F=3.42; P=0.04), and posterior cingulate (F=3.76; P=0.03). No significant effects were observed in a cortical control region. *Post hoc* testing identified greater cholinergic activity in the EPT group compared to the no-HT group in the left hippocampus (P=0.048) and posterior cingulate (P=0.045), with a non-

statistically significant trend in the right hippocampus (P=0.073).

Conclusions: A differential effect of postmenopausal ET and EPT on cholinergic neuronal integrity was identified in postmenopausal women. The findings are consistent with a preservation of cholinergic neuronal integrity in the EPT group.

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GnRH-Deficient Phenotypes in Humans and Mice with Heterozygous Variants in *KISS1/Kiss1*

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Context: KISS1 is a candidate gene for GnRH deficiency.

Objective: Our objective was to identify deleterious mutations in *KISS1*.

Patients and Methods: DNA sequencing and assessment of the effects of rare sequence variants (RSV) were conducted in 1025 probands with GnRH deficiency.

Results: Fifteen probands harbored 10 heterozygous RSV in *KISS1* seen in less than 1% of control subjects. Of the variants that reside within the mature kisspeptin peptide, p.F117L (but not p.S77I, p.Q82K, p.H90D, or p. P110T) reduces inositol phosphate generation. Of the variants that lie within the coding region but outside the mature peptide, p.G35S and p.C53R (but not p.A129V) are predicted *in silico* to be deleterious. Of the variants that lie outside the coding region, one (g.1-3659 C→T) impairs transcription in vitro, and another (c.1-7 C→T) lies within the consensus Kozak sequence. Of five probands tested, four had abnormal baseline LH pulse patterns.

In mice, testosterone decreases with heterozygous loss of *Kiss1* and *Kiss1r* alleles (wild type, 274±99, to double heterozygotes, 69±16 ng/dl; r^2=0.13; P=0.03). *Kiss1*/*Kiss1*r double-heterozygote males have shorter anogenital distances (13.0±0.2 vs. 15.6±0.2 mm at P34, P<0.001), females have longer estrous cycles (7.4±0.2 vs. 5.6± 0.2 day, P<0.01), and mating pairs have decreased litter frequency (0.59±0.09 vs. 0.71±0.06 litters/month, P<0.04) and size (3.5±0.2 vs. 5.4±0.3 pups per litter, P<0.001) compared with wild-type mice.

Conclusions: Deleterious, heterozygous *RSV* in *KISS1* exist at a low frequency in GnRH-deficient patients as well as in the general population in presumably normal individuals. As in $Kiss1^{+/-}/Kiss1r^{+/-}$ mice, heterozygous *KISS1* variants in humans may work with other genetic and/or environmental factors to cause abnormal reproductive function.

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Adipose Tissue Dysregulation in Patients with Metabolic Syndrome

Andrew A. Bremer, Sridevi Devaraj, Alaa Afify, and Ishwarlal Jialal

Context: The metabolic syndrome (MetS) is associated with increased risk of diabetes and cardiovascular disease (CVD). Numerous groups have shown increased circulating biomarkers of inflammation in MetS. However, there are scanty data on the cellular sources contributing to this low-grade inflammation.

Objective: The aim of this study was to determine the role of sc adipose tissue (SAT) biology in nascent MetS without concomitant diabetes or CVD.

Patients and Methods: Subjects with MetS and controls were recruited after informed consent. Fasting blood was collected, and SAT was obtained by biopsy.

Results: Circulating biomarkers of inflammation and insulin resistance, high-sensitivity C-reactive protein (hsCRP), IL-6, IL-1β, leptin, serum amyloid A, and retinol-binding protein-4 (RBP-4) concentrations were significantly higher in the MetS subjects than controls, whereas adiponectin concentrations were lower. In SAT, leptin, RBP-4, CRP, serum amyloid A, plasminogen activator inhibitor-1, IL-1, IL-6, IL-8, and monocyte chemotactic protein-1 (MCP-1) levels were significantly higher in MetS than controls. These differences except for RBP-4 persisted after adjusting for waist circumference. In addition, there were significantly increased numbers of macrophages infiltrating the SAT of MetS and increased numbers of crown-like structures compared with controls. hsCRP correlated positively with homeostasis model assessment and SAT MCP-1 and negatively with adiponectin. Homeostasis model assessment correlated positively with plasminogen activator inhibitor-1, RBP-4, and SAT MCP-1.

Conclusions: We make the novel observation that SAT of MetS has increased macrophage recruitment with cardinal crown-like structure features and contributes to the increased cellular inflammation that produces increased levels of biomarkers that are correlated with both insulin resistance and low-grade inflammation. These aberrations could contribute to the progression of MetS and the increased risk for diabetes and CVD.

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Catecholaminergic Axonal Varicosities Appear to Innervate Growth Hormone-Releasing Hormone-Immunoreactive Neurons in the Human Hypothalamus: The Possible Morphological Substrate of the Stress-Suppressed Growth

Giorgio Rotoli, George Grignol, Walter Hu, Istvan Merchenthaler, and Bertalan Dudas

Context: Stress is considered to be a major factor in the regulation of growth. Psychosocial dwarfism, characterized with short stature, delayed puberty, and depression, is typically preceded by psychological harassment or stressful environment. It has been observed that stress suppresses GH secretion, possibly via the attenuation of GHRH secretion. However, the exact mechanism of the impact of stress on growth has not been elucidated yet.

Objective: Our previous studies revealed intimate associations between neuropeptide Y (NPY)-immunoreactive (IR) axonal varicosities and GHRH-IR perikarya in the human hypothalamus. Because NPY is considered to be a stress molecule, NPY-GHRH juxtapositions may represent an important factor of stress-suppressed GHRH release. In addition to NPY, catecholamines are among the major markers of stress. Thus, in the present study, we examined the putative juxtapositions between the catecholaminergic tyrosine hydroxylase (TH)-/dopamine-β-hydroxylase-/phenylethanolamine *N*-methyltransferase-IR and GHRH-IR neural elements in the human hypothalamus. To reveal these juxtapositions, double-label immunohistochemistry was used.

Results: Our findings revealed that the majority of the GHRH-IR perikarya formed intimate associations with TH-IR fiber varicosities. The majority of these juxtapositions were found in the infundibular nucleus/median eminence.

Conclusions: The lack of phenylethanolamine N-methyltransferase-GHRH associations and the small number of dopamine-βhydroxylase-GHRH juxtapositions suggest that the vast majority of the observed TH-GHRH juxtapositions represent dopaminergic associations. The density of the abutting TH-IR fibers on the surface of the GHRH perikarya suggests that these juxtapositions may be functional synapses, and thus, in addition to NPY, catecholamines may regulate GHRH secretion via direct synaptic mechanisms.

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Cost-Effectiveness of a Novel Molecular Test for Cytologically Indeterminate Thyroid Nodules

Henry Li, Karen A. Robinson, Blair Anton, Ian J. Saldanha, and Paul W. Ladenson

Context: Determining which patients with thyroid nodules require surgery is limited by cytologically indeterminate findings. A new approach for preoperative molecular classification of cytologically indeterminate thyroid nodules has a reported sensitivity of 91% and specificity of 75%; however, its cost-effectiveness has yet to be assessed.

Objective: Our objective was to evaluate the 5-yr cost-effectiveness of routine use of a molecular test in adult patients with indeterminate fine-needle aspiration biopsy results from a societal perspective.

Design: A 16-state Markov decision model was developed. Probabilities, costs, and quality-adjusted life years (QALY) were estimated from literature review, U.S. Department of Health and Human Services data, Medicare reimbursement schedules, and expert opinion.

Setting and Subjects: Decision analysis of a hypothetical group of adult patients with cytologically indeterminate thyroid nodules was conducted.

Main Outcome Measures: Incremental cost-effectiveness ratio was calculated as incremental cost (measured in U.S. dollars) divided by incremental effectiveness (measured in QALY).

Results: Modifying current practice with use of the molecular test resulted in 74% fewer surgeries for benign nodules with no greater number of untreated cancers. Over 5 year, mean discounted cost estimates were $12,172 for current practice and $10,719 with the molecular test. Current practice and molecular test use produced 4.50 and 4.57 QALY, respectively.

Conclusions: Use of this novel molecular test for differential diagnosis of cytologically indeterminate thyroid nodules can potentially avoid almost three fourths of currently performed surgeries in patients with benign nodules. Compared with current practice based on cytological findings alone, use of this test may result in lower overall costs and modestly improved quality of life for patients with indeterminate thyroid nodules.

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Preoperative Insulin Resistance and the Impact of Feeding on Postoperative Protein Balance: A Stable Isotope Study

Francesco Donatelli, Davide Corbella, Marta Di Nicola, Franco Carli, Luca Lorini, Roberto Fumagalli, and Gianni Biolo Context: Major surgery induces a catabolic state resulting in a net loss of body protein.

Objectives: Our objective was to compare protein metabolism before and after surgery in nondiabetic patients with and without preoperative insulin resistance (IR). It was hypothesized that the anabolic response to feeding would be significantly impaired in those patients with preoperative insulin resistance.

Design: A hyperinsulinemic-euglycemic clamp has been used to identify two groups of patients: IR and insulin

sensitive (IS). A tracer kinetics technique has been used to evaluate the metabolic response to food intake in both groups.

Setting: Patients undergoing cardiopulmonary bypass participated.

Patients or Other Participants: Ten IS patients and 10 IR patients were enrolled in the study.

Intervention: After an overnight fasting, a 3-h infusion of a solution composed of 20% glucose and of amino acids at a rate of 0.67 and 0.44 kcal/kg·h, respectively, was started in each group. Phenylalanine kinetics were studied at the end of fasting and feeding.

Main Outcome Measure: Effect of feeding on protein balance before and after surgery was evaluated. Protein balance has been measured as the net difference of protein breakdown minus protein synthesis.

Results: Protein balance increase after postoperative feeding was blunted only in the IR group. In contrast, in the IS group, the postoperative anabolic effect of feeding was the same as before surgery.

Conclusions: These findings propose a link between insulin resistance and protein metabolism. When non-IR patients are fed, a significant anabolic effect in the postoperative period is demonstrated. In contrast, IR patients are less able to use feeding for synthetic purposes.

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Skeletal Muscle Mitochondrial Function Is Associated with Longitudinal Growth Velocity in Children and Adolescents

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Context: Periods of rapid growth require an increase in energy use and substrate formation. Mitochondrial function contributes to each of these and therefore may play a role in longitudinal growth.

Methods: Twenty-nine children and adolescents of ages 8–15 year were enrolled in a comprehensive longitudinal assessment of glucose homeostasis and mitochondrial function. Fasting laboratory studies and an estimate of mitochondrial function (as assessed by the time to recovery of phosphocreatine (PCr) concentration after submaximal quadriceps extension/flexion exercise using P magnetic resonance spectroscopy) were obtained at baseline and annually for 2 year.

Results: Data were complete for 23 subjects. Subjects were 11.3±1.9 (SD) yr old at the beginning of the study; 61% were male. Average annualized growth velocity at 1 year for boys was 7.1±1.5 cm/year and for girls 6.5±1.7 cm/year. More rapid recovery of PCr concentration, suggestive of greater skeletal muscle oxidative phosphorylation capacity at baseline, was associated with faster growth velocity in the subsequent year (R^2=0.29; P=0.008). In multivariate modeling, baseline mitochondrial function remained significantly and independently associated with growth (R^2 for model=0.51; P=0.05 for effect of PCrτ), controlling for age, gender, Tanner stage, body mass index Z-score, and height Z-score.

Conclusions: We report a novel association between time to recovery of PCr concentration after submaximal exercise and faster annual linear growth in healthy children. Future studies are needed to determine the physiological mechanisms and clinical consequences of this observation.

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Telomere Length in Neoplastic and Nonneoplastic Tissues of Patients with Familial and Sporadic Papillary Thyroid Cancer

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Introduction: Many studies have found an association between altered telomere length (TL), both attrition or elongation, and cancer phenotype. Recently, we have reported that patients with the familial form of papillary thyroid cancer (FPTC) have short telomeres in blood leucocytes.

Aim: To evaluate relative TL (RTL) at somatic level in neoplastic and nonneoplastic tissues of patients with FPTC (n=30) and sporadic PTC (n=46).

Methods: RTL was measured by quantitative PCR in neoplastic thyroid tissues, in the corresponding nontumor thyroid tissues (normal contralateral thyroid), and in other extrathyroidal tissues (lymph nodes, muscles, or buccal mucosa). RTL was also measured in adenomas and hyperplastic nodules. In a subset of samples, telomerase expression was measured by quantitative PCR.

Results: Mean±SD RTL of FPTC patients was short in neoplastic thyroid tissues (0.87±0.2) with no difference from the normal contralateral thyroid tissues (0.85±0.11) and extrathyroidal tissues (0.85±0.31). On the contrary, in patients with sporadic PTC, the mean±SD RTL in the neoplastic tissues (1.73±0.63) was significantly shorter than that found in normal contralateral tissues (2.58±0.89) and extrathyroidal tissues (2.5±0.86). For all tissue samples (cancer, normal thyroid, and nonthyroidal tissues) the mean ±SD RTL of familial cases was shorter (P<0.0001) than that found in tissues from sporadic PTC. RTL of FPTC was also lower (P<0.0001) than that of 23 follicular adenomas (1.6±0.7) and 24 hyperplastic nodules (2.2±0.9).

Conclusions: Our results demonstrate that short telomeres are a consistent feature of PTC, which in familial cases, is not restricted to the tumor tissue. This finding suggests that FPTC has a distinct, heritable, genetic background.

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A Novel Entity of Clinically Isolated Adrenal Insufficiency Caused by a Partially Inactivating Mutation of the Gene Encoding for P450 Side Chain Cleavage Enzyme (CYP11A1)

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Context: Cytochrome P450 side-chain cleavage enzyme (CYP11A1) facilitates the first and rate-limiting step of steroidogenesis. Only nine patients with CYP11A1 deficiency have been described. All patients presented with adrenal insufficiency (AI) and disorder of sex development in 46,XY individuals.

Objective: Our objective was to define the pathogenic consequences of a novel *CYP11A1* mutation (p.R451W) found in two brothers with isolated adrenal insufficiency.

Patients: The two brothers (46,XY) presented with AI and normal male genital development. The older boy first presented with signs and symptoms suggestive of AI at the age of 2.8 year but was only diagnosed at the age of 4.1 year during an adrenal crisis. The younger brother was diagnosed with AI at the age of 2.5 year while being clinically asymptomatic. Both boys had entirely normal appearance of their external genitalia.

Results: The novel p.R451W mutation and five published missense CYP11A1 mutations were characterized employing two in vitro approaches using the natural substrate cholesterol and the intermediate 22R-hydroxycholesterol, respectively. Pregnenolone generation was measured by highly specific liquid chromatography tandem mass spectrometry. p.R451W had 30% of wild-type activity consistent with the clinical phenotype in our patients. Two previously published mutations (p.L222P and p.A359V) had 2-to 3-fold higher in vitro activities than originally reported, correlating better with the associated phenotypes.

Conclusions: We provide the first evidence that partial CYP11A1 deficiency has to be considered as a differential diagnosis in clinically isolated adrenal insufficiency. Our assays demonstrate a tighter genotype-phenotype correlation in CYP11A1 deficiency than previous in vitro studies.

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Tumor Expression of Human Growth Hormone and Human Prolactin Predict a Worse Survival Outcome in Patients with Mammary or Endometrial Carcinoma

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Context: Evidence suggests that human GH (hGH) and human prolactin (hPRL) possess an autocrine or paracrine oncogenic role in mammary and endometrial carcinoma. However, especially for hGH, the prognostic relevance of tumor expression of these hormones is not well defined.

Objective: We investigated the potential association of tumor mRNA and protein expression of hGH and hPRL with the clinicopathological features of mammary and endometrial carcinoma. The prognostic relevance of the individual or combined expression of hGH and hPRL in mammary and endometrial carcinoma was also determined.

Design: The expression of hGH and hPRL was analyzed in histopathological samples of mammary and endometrial carcinoma, and the respective normal tissues, by in situ hybridization and immunohistochemistry. Kaplan-Meier and Cox regression analysis was performed to examine the association of tumor hGH and hPRL expression with relapse-free survival and overall survival of patients.

Results: hGH expression was significantly associated with lymph node metastasis, tumor stage, human epidermal growth factor receptor-2 status, and proliferative index in mammary carcinoma and with International Federation of Gynecology and Obstetrics grade, myometrial invasion, and ovarian metastases in endometrial carcinoma. hPRL expression was associated with lymph node metastasis, tumor grade, and tumor stage in mammary carcinoma and with International Federation of Gynecology and Obstetrics stage and myometrial invasion in endometrial carcinoma. Both hGH and hPRL expression, individually and combined, are associated with worse relapse-free survival and overall survival in patients with mammary or endometrial carcinoma.

Conclusion: Tumor expression of both hGH and hPRL in mammary and endometrial carcinoma is associated with a large and significant difference in survival outcome for patients with these tumors.

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Measurement of mRNA Transcripts of Very High Placental Expression in Maternal Blood as Biomarkers of Preeclampsia

Premila Paiva, Clare Whitehead, Burcu Saglam, Kirsten Palmer, and Stephen Tong

Context: mRNA of placental origin in maternal blood shows potential as a clinical biomarker of obstetric diseases such as preeclampsia (PE). We hypothesized that mRNA

transcripts very highly expressed in the placenta relative to other tissues will be differentially expressed in PE and be useful as mRNA biomarkers in maternal blood.

Objective: Our objective was to identify a panel of genes highly expressed in the placenta and compare their expression in placenta and maternal whole blood from PE vs. control pregnancies.

Setting: Placental tissue and maternal whole blood specimens were obtained from normotensive controls (n=15) and pregnancies complicated by severe preterm PE (n=21).

Intervention: mRNA expression was evaluated by quantitative real-time RT-PCR.

Results: We identified 20 genes exhibiting highest to fourth highest expression in the placenta relative to all other tissues. All genes were detectable in placenta. Nine of the 20 genes were detectable in maternal whole blood. Four of the nine genes detectable in blood (i.e. *PLAC3*, *PLAC4*, *CRH*, and *ERVWE1*) were significantly increased in both maternal blood and placenta from PE pregnancies. The remaining five genes detectable in maternal blood were unchanged in both blood and placenta from PE pregnancies. Thus, there was complete correlation of gene expression between maternal blood and placenta.

Conclusions: Circulating mRNA coding genes of high placental expression show strong correlation with transcript levels in preeclamptic placenta. Such transcripts may be promising candidates to screen as mRNA biomarkers of PE in maternal whole blood.

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Neuropilin-2 Expression in Papillary Thyroid Carcinoma: Correlation with VEGF-D Expression, Lymph Node Metastasis, and VEGF-D-Induced Aggressive Cancer Cell Phenotype

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Context: Neuropilin-2 (Nrp2) is a coreceptor for vascular endothelial growth factor-D (VEGF-D) that is expressed on the surface of endothelial cells. Recently, Nrp2 was shown to play a role in lymph node metastasis and promotion of cancer cell migration. VEGF-D also promotes lymphangiogenesis, which in turn promotes tumor metastasis.

Objective: The aim was to study the role of neuropilin-2 in lymph node metastasis in human papillary thyroid carcinoma (PTC).

Design: Expression of Nrp2 was studied by immunohistochemistry and the relationship between Nrp2 expression and lymph node metastasis, VEGF-D expression and other established clinicopathological variables were analyzed in PTC. The effects of neutralizing anti-Nrp2 antibody on VEGF-D-induced invasion and migration were assessed in PTC cell lines.

Results: Nrp2 expression was observed in 64.3% (36 of 56) of the PTC patients. Nrp2 expression was significantly correlated with lymph node metastasis (P=0.0216) and VEGF-D expression (P=0.0034). VEGF-D was shown to promote filopodia formation and cancer cell migration and invasion by K1 and B-CPAP cells. These responses were significantly blocked by neutralizing antiNrp2 antibody.

Conclusion: Nrp2 expression was correlated with lymph node metastasis and VEGF-D expression in PTC. Our data also showed a role for Nrp2 in regulating VEGF-D-induced invasion and migration in vitro.

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MicroRNAs in Thyroid Cancer

Albert de la Chapelle and Krystian Jazdzewski

Context: Traditionally, factors predisposing to diseases are either genetic (‘nature’) or environmental, also known as lifestyle-related (‘nurture’). Papillary thyroid cancer is an example of a disease where the respective roles of these factors are surprisingly unclear.

Evidence Acquisition: Original articles and reviews summarizing our current understanding of the role of microRNA in thyroid tumorigenesis are reviewed and evaluated.

Conclusion: The genetic predisposition to papillary thyroid cancer appears to consist of a variety of gene mutations that are mostly either of low penetrance and common or of high penetrance but rare. Moreover, they likely interact with each other and with environmental factors. The culpable genes may not be of the traditional, protein-coding type. A limited number of noncoding candidate genes have indeed been described, and we propose here that the failure to find mutations in traditional protein-coding genes is not coincidental. Instead, a more likely hypothesis is that changes in the expression of multiple regulatory RNA genes, e.g. miR, may be a major mechanism. Our review of the literature strongly supports this notion in that a polymorphism in one miR (miR-146a) predisposes to thyroid carcinoma, whereas numerous other miR are involved in signaling (mainly PTEN/PI3K/AKT and T3/THRB) that is central to thyroid carcinogenesis.

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Estrogen Receptor-β and Fetoplacental Endothelial Prostanoid Biosynthesis: A Link to Clinically Demonstrated Fetal Growth Restriction

Emily J. Su, Linda Ernst, Nadine Abdallah, Robert Chatterton, Hong Xin, Diana Monsivais, John Coon, and Serdar E. Bulun

Context: Fetal growth restriction (FGR) due to placental dysfunction impacts short-and long-term neonatal outcomes. Abnormal umbilical artery Doppler velocimetry indicating elevated fetoplacental vascular resistance has been associated with fetal morbidity and mortality. Estrogen receptors are regulators of vasomotor tone, and fetoplacental endothelium expresses estrogen receptor-β (ESR2) as its sole estrogen receptor.

Objective: Our objective was to elucidate the mechanism whereby ESR2 regulates placental villous endothelial cell prostanoid biosynthesis.

Design and Participants: We conducted immunohistochemical analysis of human placental specimens and studies of primary fetoplacental endothelial cells isolated from subjects with uncomplicated pregnancies.

Main Outcome Measures: We evaluated in vivo levels of ESR2 and cyclooxygenase-2 (PTGS2) in villous endothelial cells from fetuses with or without FGR and/or abnormal umbilical artery Doppler indices and in vitro effects of ESR2 on prostanoid biosynthetic gene expression.

Results: ESR2 and PTGS2 expression were significantly higher within subjects with FGR with abnormal umbilical artery Doppler indices in comparison with controls ($P<0.01$). ESR2 knockdown led to decreased cyclooxygenase-1 (PTGS1), PTGS2, prostaglandin F synthase (AKR1C3), and increased prostacyclin synthase (PTGIS), with opposing results found after ESR2 over-expression ($P<0.05$). ESR2 mediates prostaglandin H2 substrate availability and, in the setting of differential regulation of AKR1C3 and PTGIS, altered the balance between vasodilatory and vasoconstricting prostanoid production.

Conclusions: Higher ESR2 expression in the placental vasculature of FGR subjects with abnormal blood flow is associated with an endothelial cell phenotype that preferentially produces vasoconstrictive prostanoids. Endothelial ESR2 appears to be a master regulator of prostanoid biosynthesis and contributes to high-resistance fetoplacental blood flow, thereby increasing morbidity and mortality associated with FGR.

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Decreased *STAMP2* Expression in Association with Visceral Adipose Tissue Dysfunction

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Context: Six-transmembrane protein of prostate 2 (STAMP2) is a counter-regulator of inflammation and insulin resistance according to findings in mice. However, there have been contradictory reports in humans.

Objective: We aimed to explore STAMP2 in association with inflammatory and metabolic status of human obesity.

Design, Patients, and Methods: *STAMP2* gene expression was analyzed in adipose tissue samples (171 visceral and 67 sc depots) and during human preadipocyte differentiation. Human adipocytes were treated with macrophage-conditioned medium, TNF-α, and rosiglitazone.

Results: In visceral adipose tissue, *STAMP2* gene expression was significantly decreased in obese subjects, mainly in obese subjects with type 2 diabetes. *STAMP2* gene expression and protein were significantly and inversely associated with obesity phenotype measures (body mass index, waist, hip, and fat mass) and obesity-associated metabolic disturbances (systolic blood pressure and fasting glucose). In addition, *STAMP*2 gene expression was positively associated with lipogenic (*FASN*, *ACC1*, *SREBP1*, *THRSP14*, *TRα*, and *TRα1*), caveolin-1, *IRS1*, *GLUT4*, and *CD20*6 gene expression. In sc adipose tissue, *STAMP2* gene expression was not associated with metabolic parameters. In both fat depots, *STAMP2* gene expression in stromovascular cells was significantly higher than in mature adipocytes. *STAMP2* gene expression was significantly increased during the differentiation process in parallel to adipogenic genes, being increased in preadipocytes derived from lean subjects. Macrophage-conditioned medium (25%) and TNF-α (100 ng/ml) administration increased whereas rosiglitazone (2 μM) decreased significantly *STAMP2* gene expression in human differentiated adipocytes.

Conclusions: Decreased STAMP2 expression (mRNA and protein) might reflect visceral adipose dysfunction in subjects with obesity and type 2 diabetes.

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Preferential Fat Deposition in Subcutaneous Versus Visceral Depots Is Associated with Insulin Sensitivity

Tracey McLaughlin, Cindy Lamendola, Alice Liu, and Fahim Abbasi

Background: Studies on the relationship between regional fat and insulin resistance yield mixed results. Our objective was to determine whether regional fat distribution, independent of obesity, is associated with insulin resistance.

Design: Subjects included 115 healthy, overweight/moderately obese adults with body mass index (BMI) 25–36.9 kg/m^2 who met predetermined criteria for being insulin resistant (IR) or insulin sensitive (IS) based on the modified insulin suppression test. Computerized tomography was used to quantify visceral adipose tissue (VAT), sc adipose tissue (SAT), and thigh adipose tissue. Fat mass in each depot was compared according to IR/IS group, adjusting for BMI and sex.

Results: Despite nearly identical mean BMI in the IR vs. IS groups, VAT and%VAT were significantly higher in the IR group, whereas SAT,%SAT, and thigh sc fat were significantly lower. In logistic regression analysis, each SD increase in VAT increased the odds of being IR by 80%, whereas each increase in SAT decreased the odds by 48%; each increase in thigh fat decreased the odds by 59% and retained significance after adjusting for other depots. When grouped by VAT tertile, IS vs. IR individuals had significantly more SAT. There was no statistically significant interaction between sex and these relationships.

Conclusion: These data demonstrate that after adjustment for BMI and VAT mass, sc abdominal and thigh fat are protective for insulin resistance, whereas VAT, after adjustment for SAT and BMI, has the opposite effect. Whether causal in nature or a marker of underlying pathology, these results clarify that regional distribution of fat-favoring sc depots is associated with lower risk for insulin resistance.

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