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SPECIAL FEATURE

Translational Highlights

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

Insulin-Mediated Oxidative Stress and DNA Damage in LLC-PK1 Pig Kidney Cell Line, Female Rat Primary Kidney Cells, and Male ZDF Rat Kidneys in Vivo

Eman Maher Othman, Michael C. Kreissl, Franz R. Kaiser, Paula-Anahi Arias-Loza, and Helga Stopper

Hyperinsulinemia, a condition with excessively high insulin blood levels, is related to an increased cancer incidence. Diabetes mellitus is the most common of several diseases accompanied by hyperinsulinemia. Because an elevated kidney cancer risk was reported for diabetic patients, we investigated the induction of genomic damage by insulin in LLC-PK1 pig kidney cells, rat primary kidney cells, and ZDF rat kidneys. Insulin at a concentration of 5 nM caused a significant increase in DNA damage in vitro. This was associated with the formation of reactive oxygen species (ROS). In the presence of antioxidants, blockers of the insulin, and IGF-I receptors, and a phosphatidyl-inositol 3-kinase inhibitor, the insulin-mediated DNA damage was reduced. Phosphorylation of protein kinase B (PKB or AKT) was increased and p53 accumulated. Inhibition of the mitochondrial and nicotinamide adenine dinucleotide phosphatase oxidase-related ROS production reduced the insulin-mediated damage. In primary rat cells, insulin also induced genomic damage. In kidneys from healthy, lean ZDF rats, which were infused with insulin to yield normal or high blood insulin levels, while keeping blood glucose levels constant, the amounts of ROS and the tumor protein (p53) were elevated in the high-insulin group compared with the control level group. ROS and p53 were also elevated in diabetic obese ZDF rats. Overall, insulin-induced oxidative stress resulted in genomic damage. If the same mechanisms are active in patients, hyperinsulinemia might cause genomic damage through the induction of ROS contributing to the increased cancer risk, against which the use of antioxidants and/or ROS production inhibitors might exert protective effects.

This article appears in Endocrinology, published March 1, 2013, 10.1210/en.2012-1768

Nfil3 Is a Glucocorticoid-Regulated Gene Required for Glucocorticoid-Induced Apoptosis in Male Murine T Cells

Kirstyn T. Carey, Kheng H. Tan, Judy Ng, Douglas R. Liddicoat, Dale I. Godfrey, and Timothy J. Cole

Glucocorticoids (GCs) have essential roles in the regulation of development, integrated metabolism, and immune and neurological responses, and act primarily via the glucocorticoid receptor (GR). In most cells, GC treatment results in down-regulation of GR mRNA and protein levels via negative feedback mechanisms. However, in GC-treated thymocytes, GR protein levels are maintained at a high level, increasing sensitivity of thymocytes to GCs, resulting in apoptosis termed glucocorticoid-induced cell death (GICD). $CD4^+CD8^+$ double-positive thymocytes and thymic natural killer T cells in particular are highly sensitive to GICD. Although GICD is exploited via the use of synthetic GC analogues in the treatment of hematopoietic malignancies, the intracellular molecular pathway of GICD is not well understood. To explore GICD in thymocytes, the authors performed whole genome expression microarray analysis in mouse GR exon 2 null vs wild-type thymus RNA 3 hours after dexamethasone treatment. Identified and validated direct GR targets included *P21* and *Bim*, in addition to an important transcriptional regulator *Nfil3*, which previously has been associated with GICD and is essential for

natural killer cell development in vivo. Immunostaining of NFIL3 in whole thymus localized NFIL3 primarily to the medullary region, and double labeling colocalized NFIL3 to apoptotic cells. In silico analysis revealed a putative GC response element 5 kb upstream of the *Nfil3* promoter that is strongly conserved in the rat genome and was confirmed to bind GR by chromatin immunoprecipitation. The knockdown of *Nfil3* mRNA levels to 20 % of normal using specific small interfering RNAs abrogated GICD, indicating that NFIL3 is required for normal GICD in CTLL-2 T cells.

This article appears in Endocrinology, published February 20, 2013, 10.1210/en.2012-1820

Follistatin-like 3 (FSTL3) Mediated Silencing of Transforming Growth Factor β (TGFβ) Signaling Is Essential for Testicular Aging and Regulating Testis Size

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Follistatin-like 3 (FSTL3) is a glycoprotein that binds and inhibits the action of TGFβ ligands such as activin. The roles played by FSTL3 and activin signaling in organ development and homeostasis are not fully understood. The authors show mice deficient in FSTL3 develop markedly enlarged testes that are also delayed in their age-related regression. These FSTL3 knockout mice exhibit increased Sertoli cell numbers, allowing for increased spermatogenesis but otherwise showing normal testicular function. The data show that FSTL3 deletion leads to increased AKT signaling and SIRT1 expression in the testis. This demonstrates a cross-talk between TGFβ ligand and AKT signaling and leads to a potential mechanism for increased cellular survival and antiaging. The findings identify crucial roles for FSTL3 in limiting testis organ size and promoting age-related testicular regression.

This article appears in Endocrinology, published February 13, 2013, 10.1210/en.2012-1886

Lactating *Ctcgrp* Nulls Lose Twice the Normal Bone Mineral Content due to Fewer Osteoblasts and More Osteoclasts, Whereas Bone Mass Is Fully Restored After Weaning in Association With Up-Regulation of Wnt Signaling and Other Novel Genes

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The maternal skeleton resorbs during lactation to provide calcium to milk and the lost mineral content is restored after weaning. The changes are particularly marked in *Ctcgrp* null mice, which lose 50 % of spine mineral content during lactation but restore it fully. The known calciotropic hormones are not required for skeletal recovery to occur; therefore, unknown factors that stimulate bone formation may be responsible. We hypothesized that the genes responsible for regulating postweaning bone formation are differentially regulated in bone or marrow, and this regulation may be more marked in *Ctcgrp* null mice. We confirmed that *Ctcgrp* null mice had twice as many osteoclasts and 30 – 40 % fewer osteoblasts as compared with wild-type mice during lactation but no deficit in osteoblast numbers after weaning. Genome-wide microarray analyses on tibial RNA showed differential expression of 729 genes in wild-type mice at day 7 after weaning vs prepregnancy, whereas the same comparison in *Ctcgrp* null mice revealed only 283 genes. Down-regulation of Wnt family inhibitors, *Sost* and *Dkk1*, and inhibition of *Mef2c*, a sclerostin stimulator, were observed. *Ctsk*, a gene expressed during osteoclast differentiation, and *Igfbp2*, which stimulates bone resorption, were inhibited. Differential regulation of genes involved in energy use was compatible with a net increase in bone formation. The most marked changes occurred in genes not previously associated with bone metabolism. In conclusion, the postlactation skeleton shows dynamic activity with more than 700 genes differentially expressed. Some of these genes are likely to promote bone formation during postweaning by stimulating the proliferation and activity of osteoblasts, inhibiting osteoclasts, and increasing energy use.

This article appears in Endocrinology, published March 5, 2013, 10.1210/en.2012-1931

GH-Releasing Hormone Induces Cardioprotection in Isolated Male Rat Heart via Activation of RISK and SAFE Pathways

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GHRH stimulates GH synthesis and release from the pituitary and exerts direct effects in extrapituitary tissues. We have previously shown that pretreatment with GHRH reduces cardiomyocyte apoptosis and improves heart function in isolated rat hearts subjected to ischemia/reperfusion (I/R). Here, we determined whether GHRH given at reperfusion reduces myocardial reperfusion injury and investigated the molecular mechanisms involved in GHRH effects. Isolated rat hearts subjected to I/R were treated at the onset of reperfusion with: 1) GHRH; 2) GHRH+GHRH antagonist JV-1-36; 3) GHRH+mitochondrial ATP-dependent potassium channel inhibitor 5-hydroxydecanoate; 4) GHRH+mitochondrial permeability transition pore opener atractyloside; 5) GHRH+phosphoinositide 3-kinase/Akt inhibitor Wortmannin (WM); and 6) GHRH+signal transducer and activator of transcription-3 inhibitor tyrphostin-AG490 (AG490). GHRH reduced infarct size at the end of reperfusion and reverted

contractility dysfunction in I/R hearts. These effects were inhibited by either JV-1-36, 5-hydroxydecanoate, atractylosid, WM, or AG490. Western blot analysis on left ventricles showed GHRH-induced phosphorylation of either the reperfusion injury salvage kinases (RISK), phosphoinositide 3-kinase/Akt, ERK1/2, and glycogen synthase kinase-3β or signal transducer and activator of transcription-3, as part of the survivor activating factor enhancement (SAFE) pathway. GHRH-induced activation of RISK and SAFE pathways was blocked by JV-1-36, WM, and AG490. Furthermore, GHRH increased the phosphorylation of endothelial nitric oxide synthase and AMP-activated protein kinase and preserved postischemic nicotinamide adenine dinucleotide (NAD^+) levels. These results suggest that GHRH protects the heart from I/R injury through receptor-mediated mechanisms, leading to activation of RISK and SAFE pathways, which converge on mitochondria and possibly on AMP-activated protein kinase.
This article appears in Endocrinology, published February 15, 2013, 10.1210/en.2012-2064

Excess Androgen During Puberty Disrupts Circadian Organization in Female Rats
Michael T. Sellix, Zachary C. Murphy, and Michael Menaker

Circadian clocks have been described in each tissue of the hypothalamo-pituitary-ovarian axis. Although a role for the clock in the timing of ovulation is indicated, the impact of diseases that disrupt fertility on clock function or the clocks' role in the etiology of these pathologies has yet to be fully appreciated. Polycystic ovary syndrome (PCOS) is a particularly devastating endocrinopathy, affecting approximately 10 % of women at childbearing age. Common features of PCOS are a polycystic ovary, amenorrhea, and excess serum androgen. Approximately 40 % of these women have metabolic syndrome, including hyperinsulinemia, dyslipidemia, and hyperleptinemia. It has been suggested that excess androgen is a critical factor in the etiology of PCOS. We have examined the effects of androgen excess during puberty on the phase of circadian clocks in tissues of the metabolic and hypothalamo-pituitaryovarian axes. Female *period1-luciferase* (*per1-luc*) rats were exposed to androgen (5α-dihydrotestosterone [DHT]) or placebo for 4-6 weeks (short term) or 9-15 weeks (long term). As expected, DHT-treated animals gained more weight than controls and had disrupted estrous cycles. At the end of treatment, tissues, including the liver, lung, kidney, white adipose, cornea, pituitary, oviduct, and ovarian follicles, were cultured, and *per1-luc* expression in each was recorded. Analysis of *per1-luc* expression revealed that DHT exposure increased phase distribution of multiple oscillators, including ovarian follicles, liver, and adipose, and altered phase synchrony between animals. These data suggest that excess androgen during puberty, a common feature of PCOS, negatively affects internal circadian organization in both the reproductive and metabolic axes.
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PGC-1α Regulates Hepatic Hepcidin Expression and Iron Homeostasis in Response to Inflammation
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Systemic iron homeostasis is finely regulated by the liver through synthesis of the peptide hormone hepcidin (HAMP), which plays an important role in duodenal iron absorption and macrophage iron release. Clinical investigations have shown that chronic and low-grade inflammation leads to the increase of serum HAMP levels and the development of various diseases such as anemia of inflammation. However, gaps remain to fully elucidate the mechanism linking inflammation and iron dysregulation. Here we show that although inflammatory stimuli increase hepatic HAMP expression and cause systemic iron deficiency in mice, they inhibit the expression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), a transcriptional coactivator actively involved in metabolic regulation. Liver-specific overexpression of PGC-1α antagonizes lipopolysaccharide-induced HAMP expression and alleviates various pathophysiological changes similar to anemia of inflammation. Consistently, overexpression of PGC-1α in $HepG_2$ or HuH7 cells also suppresses HAMP expression and reduces iron accumulation. In contrast, knockdown of PGC-1α exaggerates LPS-induced HAMP expression and iron dysregulation. At the molecular level, PGC-1α suppresses HAMP transcription via the interaction with hepatocyte nuclear factor 4α. In addition, PGC-1α is present near hepatocyte nuclear factor 4α binding site on the proximal HAMP promoter and turns the chromatin structure into an inactive state. Our data suggest a critical role for PGC-1α in the regulation of hepatic HAMP expression and iron homeostasis under inflammatory circumstances.
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Non-Nuclear–Initiated Actions of the Estrogen Receptor Protect Cortical Bone Mass
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Extensive evidence has suggested that at least some of the effects of estrogens on bone are mediated via extranuclear estrogen receptor α signaling. However, definitive proof for

this contention and the extent to which such effects may contribute to the overall protective effects of estrogens on bone maintenance have remained elusive. Here, we investigated the ability of a 17β-estradiol (E2) dendrimer conjugate (EDC), incapable of stimulating nuclear-initiated actions of estrogen receptor α, to prevent the effects of ovariectomy (OVX) on the murine skeleton. We report that EDC was as potent as an equimolar dose of E2 in preventing bone loss in the cortical compartment that represents 80 % of the entire skeleton, but was ineffective on cancellous bone. In contrast, E2 was effective in both compartments. Consistent with its effect on cortical bone mass, EDC partially prevented the loss of both vertebral and femoral strength. In addition, EDC, as did E2, prevented the OVX-induced increase in osteoclastogenesis, osteoblastogenesis, and oxidative stress. Nonetheless, the OVX-induced decrease in uterine weight was unaltered by EDC but was restored by E2. These results demonstrate that the protection of cortical bone mass by estrogens is mediated, at least in part, via a mechanism that is distinct from the classic mechanism of estrogen action on reproductive organs.

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Nuclear Receptor LRH-1 Induces the Reproductive Neuropeptide Kisspeptin in the Hypothalamus

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The differential expression and secretion of the neuropeptide kisspeptin from neurons in the arcuate (Arc) and anteroventral periventricular (AVPV) nuclei of the hypothalamus coordinate the temporal release of pituitary gonadotropins that control the female reproductive cycle. However, the molecular basis for this differential regulation is incompletely understood. Here, we report that liver receptor homolog-1 (LRH-1), a member of the nuclear receptor superfamily, is expressed in kisspeptin neurons in the Arc but not in the AVPV in female mice. LRH-1 binds directly to the kisspeptin (*Kiss1*) promoter and stimulates *Kiss1* transcription. Deletion of LRH-1 from kisspeptin neurons in mice decreased *Kiss1* expression in the Arc, leading to reduced plasma FSH levels, dysregulated follicle maturation, and prolongation of the estrous cycle. Conversely, overexpression of LRH-1 in kisspeptin neurons increased Arc *Kiss1* expression and plasma FSH concentrations. These studies provide a molecular basis for the differential regulation of basal kisspeptin expression in Arc and AVPV neurons and reveal a prominent role for LRH-1 in hypothalamus in regulating the female reproductive axis.

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ARF Represses Androgen Receptor Transactivation in Prostate Cancer

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Androgen receptor (AR) signaling is essential for prostate cancer (PCa) development in humans. The initiation of prostate malignancy and progression to a castration-resistant stage are largely contributed by the modulation of AR activity through its coregulatory proteins. We and others previously reported that p14 alternative reading frame (ARF) expression is positively correlated with the disease progression and severity of PCa. Here, we provide evidence that p14ARF physically interacts with AR and functions as an AR corespressor in both an androgen-dependent and androgen-independent manner. Endogenous ARF (p14ARF in human and p19ARF in mouse) and AR colocalize in both human PCa cells in vitro and PCa tissues of mouse and human in vivo. Overexpression of p14ARF in PCa cells significantly attenuates the activities of androgen response region (ARR2)-probasin and prostate-specific antigen (PSA) promoters. The forced expression of p14ARF in cells resulted in a suppression of PSA and NK transcription factor locus 1 (NKX3.1) expression. Conversely, knockdown of endogenous p14ARF in human PCa cells with short hairpin RNA enhanced AR transactivation activities in a dose-dependent and p53-independent manner. Furthermore, we demonstrated that p14ARF binds to both the N-terminal domain and the ligand-binding domain of AR, and the human double minute 2 (HDM2)-binding motif of p14ARF is required for the interaction of p14ARF and AR proteins. p14ARF perturbs the androgen-induced interaction between the N terminus and C terminus of AR. Most importantly, we observed that the expression of PSA is reversely correlated with p14ARF in human prostate tissues. Taken together, our results reveal a novel function of ARF in modulation of AR transactivation in PCa.

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Gonadotropin and Sex Steroid Levels in HIV-Infected Premenopausal Women and Their Association With Subclinical Atherosclerosis in HIV-Infected and -Uninfected Women in the Women's Interagency HIV Study (WIHS)

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Background: HIV-infected women may experience prolonged amenorrhea, suggesting altered gonadotropin and sex hormone levels. However, the impact of these endocrine disruptions on atherosclerosis has not been evaluated in women living with, or at risk for, HIV infection. We investigated the association of sex

hormone and gonadotropin concentrations with subclinical atherosclerosis in HIV-infected and -uninfected premenopausal women in the Women's Interagency HIV Study.
Methods: Using B-mode ultrasound, the common carotid artery intima-media thickness and distensibility were measured once. Cycle-specific FSH, total estradiol (E2), and inhibin-B concentrations were measured in 584 (414 HIV infected, 170 HIV uninfected) women. Random concentrations of total T, dehydroepiandrosterone sulphate, and SHBG were measured in 1094 (771 HIV infected, 323 HIV uninfected) women. The endocrine analytes were measured at or before the ultrasound visit. Sex hormones, FSH, and SHBG concentrations were compared between HIV-infected and -uninfected women using nonparametric testing. Linear regression models were used to evaluate the association of sex hormones, FSH, and SHBG with carotid artery intima-media thickness and distensibility adjusted for confounders. Separate analyses were conducted by HIV status.
Results: Compared with HIV-uninfected women, E2, T, and dehydroepiandrosterone sulphate concentrations were significantly lower and SHBG was higher in HIV-infected women. Adjusted for the confounders, T was significantly positively associated with distensibility (β-estimate=.04, P=.0005) among HIV-infected women, and the magnitude of association did not differ by CD4 cell count. E2 was significantly positively associated with distensibility among HIV-infected women with CD4 count less than 350 cells/μL.
Conclusions: HIV-infected women had reduced estrogen and androgen compared with HIV-uninfected premenopausal women. T deficiency is linked with carotid artery stiffness, regardless of immune suppression, whereas E2 deficiency is linked with carotid stiffness among immunocompromised HIV-infected premenopausal women. Further research is warranted to understand the impact of endocrine dysregulation on the accelerated cardiovascular disease risk in HIV-infected women.
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Clinical and Genetic Risk Factors for Type 2 Diabetes at Early or Late Post Partum After Gestational Diabetes Mellitus

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Context: Women with a history of gestational diabetes mellitus (GDM) are at increased risk of type 2 diabetes (T2DM). However, the time to progression to diabetes differs individually.
Objective: We investigated the clinical and genetic risk factors that are associated with T2DM early or late post partum after GDM pregnancy.
Design and Setting: This was a hospital-based prospective cohort study that enrolled GDM women.
Patients and Outcome Measures: A total of 843 GDM subjects were followed for the development of T2DM. Clinical risk factors were investigated during pregnancy, 2 months post partum, and annually thereafter. GDM subjects were genotyped for 21 known T2DM-associated genetic variants, and their genotype frequencies were compared with elderly nondiabetic controls.
Results: At 2 months post partum, 105 (12.5 %) subjects had T2DM (early converters). Among the 370 remaining subjects who underwent more than 1 year of follow-up, 88 (23.8 %) had newly developed T2DM (late converters). Independent risk factors for early converters were higher prepregnancy body mass index, higher area under the curve of glucose during an antepartum oral glucose tolerance test, lower fasting insulin concentration, and decreased β-cell function. Independent risk factors for late converters were higher prepregnancy body mass index and higher glucose area under the curve. Variants in *CDKN2A/2B* and *HHEX* were associated with early conversion, whereas variants in *CDKAL1* were associated with late conversion.
Conclusions: Obesity was a risk factor for both early and late T2DM converters. However, early converters had more pronounced defects in β-cell function, which might be explained, in part, by differences in genetic predisposition.
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Serum Sex Steroid Levels and Longitudinal Changes in Bone Density in Relation to the Final Menstrual Period

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Context: The associations of serum sex steroid and FSH levels with change of bone mineral density (BMD) across the complete menopausal transition are incompletely understood.
Objective: The objective of the study was to examine the associations of annual serum levels of FSH, estradiol (E_2), T, and SHBG with the rates of bone loss in 3 phases: pretransmenopausal [baseline to 1 year before the final menstrual period (FMP)], trans-menopausal (1 year before to 2 years after the FMP), later postmenopausal (≥ 2 years after the FMP).
Design: The design of the study was a repeated-measures, mixed-effects regression.
Setting: This was a community-based observational study, with a 10-year follow-up.
Participants: A total of 720 participants of the Study of Women's Health Across the Nation Bone Study participated in the study.

Outcome Measures: Annualized lumbar spine (LS) and femoral neck (FN) BMD decline was measured.
Results: The mean annual change in BMD was slowest in pretransmenopause (0.27 %/year in FN) and fastest in transmenopause (2.16 %/year in LS). In the pretransmenopausal phase, for every doubling of FSH level, LS BMD change was faster by –0.32 %/year ($P<.0001$). In the transmenopausal phase, for every doubling of FSH level, LS BMD change was –0.35 %/year faster ($P<.0001$); for every doubling of SHBG level, LS BMD change was □0.36 %/year faster ($P<.0001$). In the later postmenopausal phase, for each doubling of the E_2 level, the LS BMD change was slower by + 0.26 %/year ($P=.049$); for each SHBG doubling, the LS BMD change was 0.21 %/year slower ($P=.048$). The FN associations were weaker and inconsistent.
Conclusions: Higher E_2 levels and lower FSH levels were associated with lower rates of LS bone loss in some but not all menopausal transition phases.
This article appears in The Journal of Clinical Endocrinology & Metabolism, published February 26, 2013, 10.1210/jc.2012-3561

Higher Levels of Physical Activity Are Associated With Lower Hypothalamic-Pituitary-Adrenocortical Axis Reactivity to Psychosocial Stress in Children
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Context: Children who undertake more physical activity (PA) not only have more optimal physical health but also enjoy better mental health. However, the pathways by which PA affects wellbeing remain unclear.
Objective: To address this question, we examined whether objectively measured daytime PA was associated with diurnal hypothalamic-pituitary-adrenocortical axis (HPAA) activity and HPAA responses to psychosocial stress.
Design and Setting: We conducted a cross-sectional study in a birth cohort in Helsinki, Finland.
Participants: We studied 258 8-year-old children.
Main Outcome Measures: PA was assessed with wrist-worn accelerometers. Overall PA and percentage of time spent in vigorous PA (VPA) were categorized by sex into thirds. Salivary cortisol was measured diurnally and in response to the Trier Social Stress Test for Children.
Results: The children in different PA groups did not show differences in diurnal salivary cortisol ($P>.10$ for overall PA and VPA). Children with the highest levels of overall PA or VPA showed no, or only small, increases over time in salivary cortisol after stress ($P=.10$ and $P=.03$ for time in analyses of PA and VPA, respectively), whereas children belonging to the lowest and intermediate thirds showed significant increases over time in salivary cortisol after stress ($P\leq.002$ for time in the analyses of overall PA and VPA).
Conclusions: These results suggest that children with lower levels of daytime PA have higher HPAA activity in response to stress. These findings may offer insight into the pathways of PA on physical and mental well-being.
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Glucagon-Like Peptide-1 (GLP-1): Effect on Kidney Hemodynamics and Renin-Angiotensin-Aldosterone System in Healthy Men
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Introduction: Glucagon-like peptide-1 (GLP-1) is an incretin hormone with multiple actions in addition to control of glucose homeostasis. GLP-1 is known to cause natriuresis in humans, but the effects on basic renal physiology are still partly unknown.
Subjects and Methods: Twelve healthy young males were examined in a randomized, controlled, double-blinded, single-day, crossover trial to evaluate the effects of 2 hours GLP-1 infusion on kidney functions. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were assessed with ^{51}Cr-EDTA and ^{123}I-hippuran, respectively, using a constant infusion renal clearance technique based on timed urine sampling.
Results: GLP-1 had no significant effect on either GFR [+ 1.9 %, 95 % confidence interval (–0.8; 4.6 %)] or RPF [+ 2.4 %, 95 % confidence interval (–3.6; 8.8 %)]. Fractional urine excretion of lithium increased 9 % ($P=.013$) and renal sodium clearance increased 40 % ($P=.007$). Angiotensin II decreased 19 % ($P=.003$), whereas renin, aldosterone, and the urinary excretion of angiotensinogen showed no significant changes. GLP-1 did not affect blood pressure but induced a small transient increase in heart rate.
Conclusion: The results indicate that although GLP-1 markedly reduces proximal tubule sodium reabsorption, the acute effects on GFR and RPF are very limited in healthy humans. The finding of GLP-1's ability to reduce angiotensin II concentration is novel and should be further elucidated.
This article appears in The Journal of Clinical Endocrinology & Metabolism, published March 5, 2013, 10.1210/jc.2012-3855

Divergences in Insulin Resistance Between the Different Phenotypes of the Polycystic Ovary Syndrome
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Context/Objective: Current diagnostic criteria for polycystic ovary syndrome (PCOS) have generated distinct PCOS phenotypes, based on the different combinations of diagnostic features found in each patient. Our aim was to assess whether either each single diagnostic feature or their combinations into the PCOS phenotypes may predict insulin resistance in these women.
Patients/Design: A total of 137 consecutive Caucasian women with PCOS, diagnosed by the Rotterdam criteria, underwent accurate assessment of diagnostic and metabolic features. Insulin sensitivity was measured by the glucose clamp technique.
Results: Among women with PCOS, 84.7 % had hyperandrogenism, 84.7 % had chronic oligoanovulation, and 89 % had polycystic ovaries. According to the individual combinations of these features, 69.4 % of women had the classic phenotype, 15.3 % had the ovulatory phenotype, and 15.3 % had the normoandrogenic phenotype. Most subjects (71.4 %) were insulin resistant. However, insulin resistance frequency differed among phenotypes, being 80.4 %, 65.0 %, and 38.1 %, respectively, in the 3 subgroups ($P<.001$). Although none of the PCOS diagnostic features per se was associated with the impairment in insulin action, after adjustment for covariates, the classic phenotype and, to a lesser extent, the ovulatory phenotype were independently associated with insulin resistance, whereas the normoandrogenic phenotype was not. Metabolic syndrome frequency was also different among phenotypes ($P=.030$).
Conclusions: There is a scale of metabolic risk among women with PCOS. Although no single diagnostic features of PCOS are independently associated with insulin resistance, their combinations, which define PCOS phenotypes, may allow physicians to establish which women should undergo metabolic screening. In metabolic terms, women belonging to the normoandrogenic phenotype behave as a separate group.
This article appears in The Journal of Clinical Endocrinology & Metabolism, published March 8, 2013, 10.1210/jc.2012-3908

Serum Parathyroid Hormone in Relation to All-Cause and Cardiovascular Mortality: The Hoorn Study

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Context: Higher PTH concentrations have been associated with fatal cardiovascular diseases (CVDs), but data in the general population are scarce.
Objective: We investigated whether higher PTH concentrations are prospectively associated with all-cause and CVD mortality.
Design, Setting, Participants: This study used data from the Hoorn Study, a prospective population-based cohort with baseline measurements between 2000 and 2001. We included 633 participants, mean age 70.1±6.6 years, 51 % female. Serum intact PTH was measured using a 2-site immunoassay.
Main Outcome Measures: Outcomes were all-cause and CVD mortality based on clinical files and coded according to the International Classification of Diseases, ninth revision. We used Kaplan-Meier plots to estimate survival curves and Cox regression to estimate hazard ratios (HRs) using season-specific PTH quartiles.
Results: During a median follow-up of 7.8 years, 112 participants died, of which 26 deaths (23 %) were cardiovascular. Survival curves by PTH quartiles differed for all-cause mortality (log-rank $P=.054$) and CVD mortality (log-rank $P=.022$). In a multivariate model, the highest PTH quartile was associated with all-cause mortality; HR=1.98 (1.08, 3.64). Kidney function slightly attenuated the PTH risk association, but risk persisted; HR=1.93 (1.04, 3.58). The results for CVD mortality showed a similar pattern, although the association was significant only in a threshold model (quartile 4 vs quartile 1–3); HR=2.56 (1.11, 5.94).
Conclusions: Among a general older population, higher PTH concentrations were associated with higher all-cause mortality risk, mostly explained by fatal CVD events. We suggest to evaluate whether individuals with high PTH concentrations benefit from therapeutic approaches targeted to decrease PTH concentrations.
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Aquaporin-1 Plays a Crucial Role in Estrogen-Induced Tubulogenesis of Vascular Endothelial Cells

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Context: Aquaporin-1 (AQP1) has been proposed as a mediator of estrogen-induced angiogenesis in human breast cancer and endometrial cancer. Elucidation of the molecular mechanisms governing AQP1-mediated, estrogen-induced angiogenesis may contribute to an improved understanding of tumor development.
Objective: Our objective was to identify the estrogen-response element (ERE) in the promoter of the *Aqp1* gene and investigate the effects and mechanisms of AQP1 on estrogen-induced tubulogenesis of vascular endothelial cells.
Setting: The study was conducted in a university hospital in eastern China.
Main Outcome Measures: Immunohistological, real-time PCR and Western blot analyses were used to determine the expression AQP1 mRNA and protein in vascular endothelial cells. Chromatin immunoprecipitation analyses and luciferase

reporter assays identified ERE-like motif in the promoter of the *Aqp1* gene.
Results: Expression of AQP1 in blood vessels of human breast and endometrial carcinoma tissues were significantly higher than controls. Estradiol (E_2) dose-dependently increased the expression levels of AQP1 mRNA and protein in human umbilical vein endothelial cells (HUVECs). A functional ERE-like motif was identified in the promoter of the *Aqp1* gene. AQP1 colocalized with ezrin, a component of the ezrin/radixin/moesin protein complex, and, ezrin colocalized with filamentous actin in HUVECs. Knockdown of AQP1 or ezrin with specific small interfering RNA significantly attenuated the formation of transcytoplasmic filamentous actin stress fibers induced by E_2 and inhibited E_2-enhanced cell proliferation, migration, invasion, and tubule formation of HUVECs.
Conclusions: Estrogen induces AQP1 expression by activating ERE in the promoter of the *Aqp1* gene, resulting in tubulogenesis of vascular endothelial cells. These results provide new insights into the molecular mechanisms underpinning the angiogenic effects of estrogen.
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Elevated Endothelin-1 (ET-1) Levels May Contribute to Hypoadiponectinemia in Childhood Obesity
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Context: Pediatric obesity is associated with endothelial dysfunction and hypoadiponectinemia, but the relationship between these two conditions remains to be fully clarified. Whether enhanced release of endothelin-1 (ET-1) may directly impair adiponectin (Ad) production in obese children is not known.
Objective: The aim of the study was to explore whether and how high circulating levels of ET-1 may contribute to impair Ad production, release, and vascular activity.
Design and Participants: Sixty children were included into obese (Ob; n=30), overweight (OW; n=11), and lean (n=19) groups. Total and high-molecular-weight Ad, ET-1, vascular cell adhesion molecule-1, and von Willebrand factor levels were measured in serum samples. Adipocytes were stimulated with exogenous ET-1 or with sera from lean, OW, and Ob, and Ad production and release measured in the absence or in the presence of ET_A (BQ123) and ET_B (BQ-788) receptor blockers, p42/44 MAPK inhibitor PD-98059, or c-Jun NH_2-terminal protein kinase inhibitor SP600125. Vasodilation to Ad was evaluated in rat isolated arteries in the absence or in the presence of BQ-123/788.
Results: Total and high-molecular-weight Ad was significantly decreased and ET-1 levels significantly increased in OW ($P<.01$) and Ob ($P<.001$) children. A statistically significant linear regression ($P<.01$) was found between Ad and ET-1. Exposure of adipocytes to exogenous ET-1 or serum from OW and Ob significantly decreased Ad mRNA and protein levels ($P<0.001$). The inhibitory effect of ET-1 on Ad was reverted by BQ-123/788 or PD-98059 but not SP-600125. Ad-mediated vasodilation was further increased in arteries pretreated with BQ-123/788.
Conclusions: ET-1-mediated inhibition of Ad synthesis via p42/44 MAPK signaling may provide a possible explanation for hypoadiponectinemia in pediatric obesity and contribute to the development of cardiovascular complications.
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Cancer Protection Elicited by a Single Nucleotide Polymorphism Close to the Adrenomedullin Gene
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Context: The risk of developing cancer is regulated by genetic variants, including polymorphisms. Characterizing such variants may help in developing protocols for personalized medicine.
Objective: Adrenomedullin is a regulatory peptide involved in cancer promotion and progression. Carriers of a single nucleotide polymorphism (SNP) in the proximity of the adrenomedullin gene have lower levels of circulating peptide. The aim of the present work was to investigate whether carriers of this SNP (rs4910118) are protected against cancer.
Design: This was a retrospective study. DNA samples were obtained from the Carlos III DNA National Bank (University of Salamanca, Salamanca, Spain).
Setting: Samples represent a variety of donors and patients from Spain.
Patients or Other Participants: DNA from patients with breast cancer (n=238), patients with lung cancer (n=348), patients with cardiac insufficiency (n=474), and healthy donors of advanced age (n=500) was used.
Interventions: All samples were genotyped using double-mismatch PCR, and confirmation was achieved by direct sequencing.
Main Outcome Measures: The minor allele frequency was calculated in all groups. The Pearson χ^2 was used to compare SNP frequencies.
Results: Of 1560 samples, 14 had the minor allele, with a minor allele frequency in healthy donors of 0.90 %. Patients with cancer had a statistically significantly lower frequency than healthy donors (odds ratio=0.216, 95 % confidence interval=0.048– 0.967, $P=.028$).

Conclusions: Carriers of the minor allele have a 4.6-fold lower risk of developing cancer than homozygotes for the major allele. Knowledge of the rs4910118 genotype may be useful for stratifying patients in clinical trials and for designing prevention strategies.
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A Novel Point Mutation in the DNA-Binding Domain (DBD) of the Human Glucocorticoid Receptor Causes Primary Generalized Glucocorticoid Resistance by Disrupting the Hydrophobic Structure of its DBD
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Context: Primary generalized glucocorticoid resistance is a rare genetic condition characterized by partial end-organ insensitivity to glucocorticoids. Most affected subjects present with clinical manifestations of mineralocorticoid and androgen excess. The condition has been associated with inactivating mutations in the human glucocorticoid receptor (hGR) gene, which impair the molecular mechanisms of hGRα action, thereby reducing tissue sensitivity to glucocorticoids.
Objective: The aim of our study was to investigate the molecular mechanisms through which one previously described natural heterozygous V423A mutation, the second mutation detected in the DNA-binding domain (DBD) of the hGRα, affects glucocorticoid signal transduction.
Design and Results: Compared with the wild-type receptor, hGRαV423A demonstrated a 72 % reduction in its ability to trans-activate the glucocorticoid-inducible mouse mammary tumor virus promoter in response to dexamethasone. The hGRαV423A receptor showed a significant reduction in its ability to bind to glucocorticoid-response elements of glucocorticoid-responsive genes, owing to structural alterations of the DBD confirmed by computer-based structural analysis. In addition, hGRαV423A demonstrated a 2.6-fold delay in nuclear translocation following exposure to the ligand, although it did not exert a dominant negative effect on the wild-type hGRα, had a similar affinity to the ligand with the wild-type receptor, and displayed a normal interaction with the GRIP1 coactivator in vitro.
Conclusions: The natural mutant receptor hGRαV423A causes primary generalized glucocorticoid resistance by affecting multiple steps in the cascade of glucocorticoid receptor action, which primarily involve decreased ability to bind to target glucocorticoid response elements and delayed translocation into the nucleus.
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Nephrogenic Diabetes Insipidus: Essential Insights into the Molecular Background and Potential Therapies for Treatment
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The water channel aquaporin-2 (AQP2), expressed in the kidney collecting ducts, plays a pivotal role in maintaining body water balance. The channel is regulated by the peptide hormone arginine vasopressin (AVP), which exerts its effects through the type 2 vasopressin receptor (AVPR2). Disrupted function or regulation of AQP2 or the AVPR2 results in nephrogenic diabetes insipidus (NDI), a common clinical condition of renal origin characterized by polydipsia and polyuria. Over several years, major research efforts have advanced our understanding of NDI at the genetic, cellular, molecular, and biological levels. NDI is commonly characterized as hereditary (congenital) NDI, arising from genetic mutations in the AVPR2 or AQP2; or acquired NDI, due to for example medical treatment or electrolyte disturbances. In this article, we provide a comprehensive overview of the genetic, cell biological, and pathophysiological causes of NDI, with emphasis on the congenital forms and the acquired forms arising from lithium and other drug therapies, acute and chronic renal failure, and disturbed levels of calcium and potassium. Additionally, we provide an overview of the exciting new treatment strategies that have been recently proposed for alleviating the symptoms of some forms of the disease and for bypassing G protein-coupled receptor signaling.
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